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REVIEW ARTICLE

The nickel ion bioavailability model of the carcinogenic potential of nickel-containing substances in the lung

Julie E. Goodman,[1] Robyn L. Prueitt,[1] Sagar Thakali,[1] and Adriana R. Oller[2]

[1]Gradient, Cambridge, Massachusetts, USA, and [2]NiPERA, Durham, North Carolina, USA

Abstract
The inhalation of nickel-containing dust has been associated with an increased risk of respiratory cancer in workplaces that process and refine sulfidic nickel mattes, where workers are exposed to mixtures of sulfidic, oxidic, water-soluble, and metallic forms of nickel. Because there is great complexity in the physical and chemical properties of nickel species, it is of interest which specific nickel forms are associated with carcinogenic risk. A bioavailability model for tumor induction by nickel has been proposed, based on the results of animal inhalation bioassays conducted on four nickel-containing substances. The nickel ion bioavailability model holds that a nickel-containing substance must release nickel ions that become bioavailable at the nucleus of epithelial respiratory cells for the substance to be carcinogenic, and that the carcinogenic potency of the substance is proportional to the degree to which the nickel ions are bioavailable at that site. This hypothesis updates the nickel ion theory, which holds that exposure to any nickel-containing substance leads to an increased cancer risk. The bioavailability of nickel ions from nickel-containing substances depends on their respiratory toxicity, clearance, intracellular uptake, and both extracellular and intracellular dissolution. Although some data gaps were identified, a weight-of-evidence evaluation indicates that the nickel ion bioavailability model may explain the existing animal and in vitro data better than the nickel ion theory. Epidemiological data are not sufficiently robust for determining which model is most appropriate, but are consistent with the nickel ion bioavailability model. Information on nickel bioavailability should be incorporated into future risk assessments.

Keywords: Mechanism of action, metals, respiratory cancer, risk assessment, tumor induction, weight of evidence

Contents

Address for Correspondence: Julie E. Goodman, Gradient, 20 University Road, Cambridge, MA 02138, USA. E-mail: jgoodman@gradientcorp.com

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1. Introduction

The inhalation of nickel-containing dust has been associated with an increased risk of respiratory cancer in workplaces that process and refine sulfidic nickel mattes, where workers experienced high exposures to mixtures of sulfidic, oxidic, water-soluble, and metallic forms of nickel. Some of these nickel substances, in different combinations, also exist outside of these workplaces. For example, nickel oxide and metallic nickel may be present together in alloy manufacturing. With regard to the carcinogenic risk of nickel substances outside of the refineries that process and refine sulfidic nickel mattes, many studies of alloy workers exist, but there are very few studies of workers in other nickel-producing or -using industries. Because of this, and also because of the complexity in the physical and chemical properties of the various forms of nickel, it is of interest to better understand which specific nickel forms are carcinogenic and the best way to quantify their risks.

The nickel ion theory has been proposed as a model for the determination of the carcinogenicity of nickel-containing substances. The theory is actually a hypothesis, in that it refers to a conjecture proposed as a possible explanation for a phenomenon, but because it is commonly referred to as a theory in the scientific literature, we use this terminology here. This theory postulates that the nickel ion is carcinogenic, and if it can be released from a nickel-containing substance, then that substance should be considered carcinogenic, as well. Based on this principle, the more nickel ion a substance releases, the higher its carcinogenic potency. Because it is expected that high water solubility results in an increased release of nickel ions, one could extrapolate from this theory that water-soluble nickel compounds are the most potent carcinogens of all nickel-containing substances.

The data supporting the nickel ion theory in 1990 included evidence that each form of nickel appeared to be weakly genotoxic in vitro (as reviewed by Goodman et al., 2009). In addition, early animal carcinogenicity studies indicated that parenteral exposures to various forms of nickel were associated with an increased local tumor incidence. Despite this, some investigators at the time recognized that the physicochemical characteristics of nickel-containing substances could affect their carcinogenic potencies (Pott et al., 1992; Sunderman and Maenza, 1976; Sunderman et al., 1987). Many regulatory and scientific bodies postulated that even if the nickel ion is the ultimate carcinogen, the carcinogenic potential of various forms of nickel substances may depend on the ability of each substance to deliver the nickel ion to the nucleus of target cells (CARB, 1991; Fletcher et al., 1994; Hansen and Stern, 1983, 1984; WHO, 1991; US EPA, 1986).

In 1996, the National Toxicology Program (NTP) completed animal inhalation bioassays with three different nickel compounds. The results of these studies did not support the nickel ion theory. The compound with the highest solubility, nickel sulfate hexahydrate ($NiSO_4 \cdot 6H_20$), showed no evidence of carcinogenicity in male and female rats and mice. The compound with intermediate solubility, nickel subsulfide (Ni_3S_2), showed clear evidence of carcinogenicity in rats at exposure levels of 0.1 mg Ni/m^3 and above. The compound with the lowest solubility, nickel oxide (NiO), showed some evidence of carcinogenicity in rats at exposure levels of 1.0 mg Ni/m^3 and above and equivocal evidence in female mice.

Since the results of the NTP bioassays were published, many others shared the view that it is the bioavailability of the nickel ion that contributes to the carcinogenicity of nickel-containing substances (Costa et al., 2003; Goodman et al., 2009; Haber et al., 2000; Hack et al., 2007; Oller, 2002; Oller et al., 1997, 2008). The specific chemical form of nickel determines whether the free nickel ion can reach the target cell nucleus and cause cancer. Some forms of nickel enhance this delivery (e.g., nickel

subsulfide); other forms allow delivery, but much less efficiently (e.g., nickel oxides); and other forms do not appear to deliver nickel ions to the nucleus in sufficient amounts to cause cancer (e.g., nickel sulfate hexahydrate and metallic nickel). Thus, the issue is not whether the nickel ion can be released from a nickel-containing substance, but whether biological conditions in the respiratory tract will allow the nickel ion to reach the nucleus of target cells in sufficient amounts to induce tumors.

Below, we describe a refinement of the nickel ion theory that represents a transition from the view that all nickel-containing substances are carcinogenic to a model of the carcinogenicity of nickel-containing substances that is based on the bioavailability of the nickel ion at nuclear sites of target respiratory epithelial cells. We discuss the animal and mechanistic data that support such a model. We also consider whether the existing epidemiological data are consistent with this model or with the nickel ion theory, and we identify remaining data gaps.

2. The nickel ion bioavailability model

The nickel ion bioavailability model is a refinement of the nickel ion theory, and it holds that the presence of nickel ions in a substance is not sufficient for that substance to be a complete carcinogen. Instead, after inhalation, a substance must release nickel ions that become bioavailable at the nucleus of epithelial respiratory cells for that substance to be carcinogenic. The carcinogenic potency of the substance releasing the nickel ions will be proportional to the degree to which nickel ions are bioavailable. The evidence from animal bioassays with individual nickel substances strongly suggests that the nickel ion bioavailability model is more scientifically supportable than the nickel ion theory for determining the carcinogenicity of a nickel-containing substance. Although this model likely predicts both lung and nasal cancer risks in humans, because nasal tumors have not been observed in animal bioassays, this paper is focused only on lung cancer. In the future, the nickel ion bioavailability model may allow the prediction of relative cancer risk for those nickel substances without available animal bioassays.

Contrary to what can be extrapolated from the nickel ion theory, it is clear that water solubility alone does not determine nickel ion uptake or release from nickel-containing substances in vivo and, therefore, does not determine nickel ion bioavailability. Data from numerous animal and mechanistic studies indicate that the bioavailability of the nickel ion at nuclear sites of target epithelial cells depends upon the interaction of many different factors—such as respiratory toxicity, clearance, target cell uptake, and intracellular dissolution—and these factors differ among the various forms of nickel (Figure 1). The intrinsic respiratory toxicity and clearance rate of a nickel-containing substance will determine the maximum retained dose after inhalation and whether an initiated cell will survive. Uptake of retained nickel particles into epithelial cells of the respiratory tract can occur by multiple mechanisms that depend on the solubility and other physicochemical characteristics of the particles. Once inside target cells, the amount of nickel ions released from nickel-containing particles by dissolution also depends on their physical and chemical properties. It is only if the nickel ion reaches the nucleus in sufficient amounts, and the cell survives, that it can ultimately lead to carcinogenesis. This implies the existence of a threshold for the initiation of carcinogenicity, even if the effects of the nickel ion in the nucleus are assumed to be genotoxic. Such a “practical” threshold for carcinogenesis is likely for certain compounds, particularly for weakly genotoxic agents where secondary mechanisms of carcinogenesis may be important (Bolt and Huici-Montagud, 2008; Hengstler et al., 2003).

The mechanism of action for nickel carcinogenesis is not known. Because nickel ions are only weakly genotoxic, other mechanisms for nickel carcinogenicity that do not require nickel ions in the nucleus have been proposed. As discussed below, some of these mechanisms describe potential tumor-promoting effects of nickel, some of which may not be nickel specific and do not support nickel as a complete carcinogen. The focus of this paper, however, is to describe a model for the determination of the complete carcinogenicity of nickel-containing substances, and the dominant mechanism for this is explained by the nickel ion bioavailability model. The animal and mechanistic data that support the nickel ion bioavailability model are described below.

3. Animal carcinogenicity studies

Unlike studies using other exposure routes, inhalation studies encompass all of the factors that contribute to nickel bioavailability at nuclear sites of target epithelial cells in the respiratory tract, which is the target organ for nickel carcinogenicity in humans and animals (Oller, 2002). Although a few inhalation animal studies were available on the carcinogenic potential of inhaled nickel compounds—including sulfidic, oxidic, and metallic nickel—prior to 1990 (Hueper, 1958; Hueper and Payne, 1962; Ottolenghi et al., 1975; Wehner et al., 1984; Tanaka et al., 1988; see Table 1), none had been conducted with water-soluble nickel compounds. Ottolenghi et al. (1975) reported an increased incidence of lung tumors in F344/N rats exposed to 1 mg/m^3 nickel subsulfide for 2 years. In contrast, nickel oxide did not increase the incidence of lung tumors in hamsters exposed to 53 mg Ni/m^3 in a 2-year study (Wehner et al., 1984). Similarly, Tanaka et al. (1988) did not observe an increased incidence of tumors in rats following nickel oxide exposures. Prior studies of metallic nickel were generally compromised by high exposures, resulting in high toxicity, and a lack of proper controls (Hueper, 1958; Hueper and Payne, 1962; see Table 1 and review by Sivulka, 2005). Nonetheless, there was generally no evidence of carcinogenicity in animals for metallic nickel via inhalation exposures.

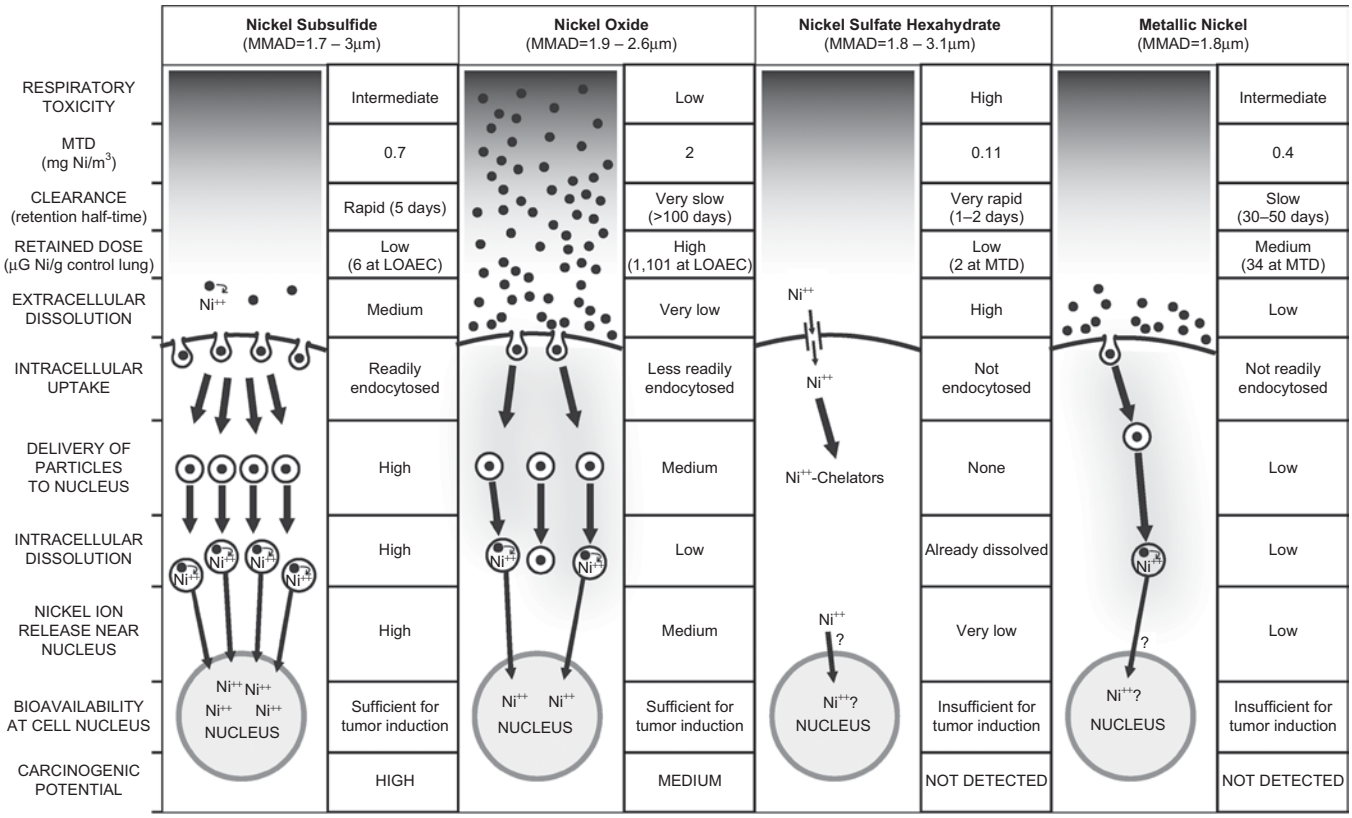

Figure 1. The nickel ion bioavailability model takes into account the various factors that determine the bioavailability of the nickel ion at the nucleus of target cells *in vivo*. The examples in this figure are based on the results of *in vitro* and *in vivo* mechanistic studies as well as the rat inhalation bioassays with nickel-containing substances.

The most robust chronic inhalation carcinogenicity studies of nickel compounds and metallic nickel were conducted by the National Toxicology Program (NTP, 1996a, 1996b, 1996c) and Oller et al. (2008), respectively. In the NTP bioassays, F344 rats and B6C3F$_1$ mice were treated with nickel subsulfide, high-temperature green nickel oxide, and nickel sulfate hexahydrate (NTP, 1996a, 1996b, 1996c; also presented in Dunnick et al., 1995). In the Oller et al. (2008) bioassay, Wistar rats were treated with nickel metal powder. Compared to the pre-1990 bioassays, the NTP (1996a, 1996b, 1996c) and Oller et al. (2008) studies represent the state-of-the-art animal inhalation studies for evaluating the carcinogenicity of inhaled nickel-containing particles.

In the NTP bioassays, 50 males and 50 females of each species were exposed to three different nickel compounds for 6 hours per day, 5 days per week, for 2 years. Exposure concentrations ranged from 0.03 to 2.0 mg Ni/ m^3 in rats and 0.06 to 3.9 mg Ni/m^3 in mice (specific dose regimens differed by compound, see Table 1) (Dunnick et al., 1995). The NTP studies were compliant with Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations and all aspects of the studies were subjected to retrospective quality assurance audits (NTP, 1996a, 1996b, 1996c). These studies examined all major organs and included chronic exposure concentrations designed to be at or below the maximum tolerable exposure concentrations in air (Haber et al., 2000). For nickel subsulfide, there was clear evidence of respiratory carcinogenicity in rats at exposure concentrations of 0.11 and 0.73 mg Ni/m^3 in males and at 0.73 mg Ni/m^3 in females, based on increases in alveolar/bronchiolar adenomas and carcinomas, but no evidence of carcinogenicity in male and female mice (NTP, 1996b). Thus, the lowest observable adverse effect concentration (LOAEC) for tumor induction in this study was 0.11 mg Ni/m^3. For nickel oxide, there was "some evidence" of respiratory carcinogenicity in rats based on increased incidences of alveolar/bronchiolar adenomas or carcinomas at 1.0 and 2.0 mg Ni/m^3 in males and 1.0 mg Ni/m^3 in females, but no evidence in male mice and equivocal evidence in female mice (NTP, 1996c). The LOAEC for tumors in this study was 1.0 mg Ni/m^3. Nasal tumors were not observed in the nickel subsulfide and nickel oxide studies, even though dosimetric calculations predict ~50% aerosol deposition in the extrathoracic region (Oller and Oberdörster, 2010). For nickel sulfate hexahydrate, there was no evidence of carcinogenicity in either species, as no exposure-related tumors were observed in the lungs or other tissues in rats (at concentrations up to 0.11 mg Ni/m^3) or mice (NTP, 1996a); thus, the no observable adverse effect concentration (NOAEC) for tumor induction in this study was 0.11 mg Ni/m^3.

Oller et al. (2008) conducted an inhalation carcinogenicity study with nickel metal powder in compliance with GLP regulations and the Organisation for Economic

Table 1. Chronic inhalation carcinogenicity studies of nickel in animals.

Form of nickel	Species	Sex, group size	Particle size[a]	Exposure concentrations (mg Ni/m^3)	Exposure protocol	Increased lung tumor incidence	Other tumors	Reference
Sulfidic nickel								
Nickel subsulfide	F344/N rats	50M, 50F	MMAD = 1.7–3.0 µm; GSD = 1.6–2.9 µm	0, 0.11, or 0.73	6 h/d, 5 d/wk, 2 yr	Adenomas and carcinomas in males at 0.73 mg Ni/m^3. Combined adenomas and carcinomas in males at 0.11 and 0.73 mg Ni/m^3 and females at 0.73 mg Ni/m^3.	Malignant pheochromocytomas in males at 0.73 mg Ni/m^3. Benign and combined pheochromocytomas in males at 0.11 and 0.73 mg Ni/m^3 and females at 0.73 mg Ni/m^3.	NTP (1996b)
	$B6C3F_1$ mice			0, 0.44, or 0.9		None	None	
Nickel subsulfide	F344 rats	108–110 M, 98–107 F	70% < 1 µm; 25% 1–1.5 µm diameter	0.97	6 h/d, 5 d/wk, 78 wk, + 30 wk observation	Lung tumors incidence: 14% in exposed rats vs. 1% in controls.	Not reported	Ottolenghi et al. (1975)
Oxidic nickel								
High-temperature green nickel oxide	F344/N rats	50M, 50F	MMAD = 1.9–2.6 µm; GSD = 1.6–2.1 µm	0, 0.5, 1.0, or 2.0	6 h/d, 5 d/wk, 2 yr	Carcinomas in females at 1.0 mg Ni/m^3, but not at 2.0 mg Ni/m^3. Combined adenomas and carcinomas in males at 1.0 and 2.0 mg Ni/m^3.	Malignant and combined pheochromocytomas in males at 2.0 mg Ni/m^3. Benign pheochromocytomas in females at 2.0 mg Ni/m^3.	NTP (1996c)
	$B6C3F_1$ mice			0, 1.0, 2.0, or 3.9		Adenomas in females at 2.0 mg Ni/m^3, but not at 3.9 mg Ni/m^3. Combined adenomas and carcinomas in females at 1.0 mg Ni/m^3, but not at 2.0 or 3.9 mg Ni/m^3.	None	
Nickel oxide	Syrian golden hamsters	51M	Median diameter = 0.3 µm; GSD = 2.2 µm	53.2	7 h/d, 5 d/wk, lifespan	None	None	Wehner et al. (1984)
"Green" nickel oxide	Wistar rats	4–5M (12 mos.);1–5M (12 mos. + 8 mos. observation)	MMAD = 0.6 µm; GSD = 1.6 µm	0.3 or 1.2	7 h/d, 5 d/wk, up to 12 mo + 8 mo clearance period	None	Not reported	Tanaka et al. (1988)
Water-soluble nickel								
Nickel sulfate hexahydrate	F344/N rats	50M, 50F	MMAD = 1.8–3.1 µm; GSD = 1.6–2.9 µm	0.03, 0.06, or 0.11	6 h/d, 5 d/wk, 2 yr	None	None	NTP (1996a)
	$B6C3F_1$ mice			0.06, 0.11, or 0.22		None	None	

Metallic nickel								
Metallic nickel	Wistar rats	50M, 50F	MMAD = 1.8 µm; GSD = 2.4 µm	0, 0.1, 0.4, and 1.0	6 h/d, 5 d/wk, up to 24 mo + 6 mo observation	None	Adrenal gland pheochromocytomas in males at 0.4 mg Ni/m^3. Combined cortical adenomas/carcinomas in females at 0.4 mg Ni/m^3.	Oller et al. (2008)
Metallic nickel	Bethesda black rats	Unknown	1–3 µm	Unknown	24 mo	None	None	Hueper and Payne (1962)
	Hamsters					None	None	
Metallic nickel	Wistar & and Bethesda black rats	50M, 110F	Majority ≤ 4 µm	15	6 h/d, 4–5 d/wk, 24 mo	None	None	Hueper (1958)
	Strain 13 guinea pigs	32M, 10F			6 h/d, 4–5 d/wk, 21 mo	None	None	
	C57 black mice	20F			6 h/d, 4–5 d/wk, 15 mo	None	None	

[a]Particle size (or, aerosol size) metrics include mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). For the NTP studies, the range of these values across exposure groups is reported.

Co-operation and Development (OECD) 451 guidelines. Fifty male and 50 female Wistar rats were exposed to nickel metal powder at concentrations ranging from 0.1 to 1.0 mg Ni/m^3. Animals were exposed for 6 hours per day, 5 days per week, for up to 24 months, followed by a 6-month period of observation. The findings were peer-reviewed and a panel of regulatory, academic, and industrial experts oversaw the study. High mortality rates among rats exposed to 1.0 mg Ni/m^3 resulted in an early termination of exposures in that group, and no exposure-related increase in tumors was observed anywhere in the respiratory tract of the animals at exposure concentrations up to 0.4 mg Ni/m^3, the NOAEC for tumors in this study (Oller et al., 2008).

The particle size of nickel compounds determines the deposition fraction in the respiratory tract of rats and humans. The particle sizes of the aerosols used in the animal inhalation bioassays were quite small, with most measures near a mass median aerodynamic diameter (MMAD) of 2 µm (see Table 1). The human deposition characteristics of aerosols indicate that virtually all of these particles would be of respirable size in humans (Hsieh et al., 1999; Oller and Oberdörster, 2010). Nickel-containing aerosols with much larger particles (e.g., >20 µm MMAD), such as those in workplaces, contain a relatively smaller proportion of respirable-size nickel (Hsieh et al., 1999). As described below, human occupational exposure concentrations were often much higher than those tested in the inhalation bioassays, but resulted in equivalent exposures to respirable-size nickel as in the animal bioassays (i.e., resulted in the same deposited dose in the pulmonary region per unit of surface area).

It should also be noted that in the animal inhalation bioassays with nickel subsulfide, nickel oxide, and nickel metal powder, increases in adrenal gland pheochromocytomas were observed. These tumors, which have also been observed in inhalation studies with talc and other compounds, were considered secondary to the respiratory toxicity and hypoxemia caused by the particulate nickel exposures (Dunnick et al., 1995; Oller et al., 2008; Ozaki et al., 2002). These tumors were not considered to be nickel ion induced, as they were not observed in animals exposed to nickel sulfate hexahydrate by inhalation (NTP, 1996a). They were also not observed in a robust oral carcinogenicity study in F344 rats of nickel sulfate hexahydrate (Heim et al., 2007). In this study, no treatment-related increases in tumor incidence were observed for any type of tumor. Nickel sulfate hexahydrate is readily solubilized in gastrointestinal fluid, resulting in a higher systemic absorption of nickel ions compared to less-soluble nickel containing substances (Ishimatsu et al., 1995; Hayman et al., 1984). This leads to the highest potential internal exposure to nickel ions and the highest potential for systemic carcinogenicity after oral exposure. Thus, these animals had much higher levels of nickel ions in their adrenal glands, yet did not develop tumors. Adrenal gland tumors have also not been observed in any of the epidemiological studies of nickel workers.

In summary, robust chronic animal inhalation carcinogenicity studies have been conducted with sulfidic, oxidic, water-soluble, and metallic nickel (NTP, 1996a, 1996b, 1996c; Oller et al., 2008). These studies indicated that the rat (but not the mouse or the hamster) is a sensitive model for the lung carcinogenicity of nickel substances. Water-insoluble nickel subsulfide was clearly carcinogenic (consistent with risks in humans, but not mice or hamsters), whereas evidence for nickel oxide was equivocal. There was no evidence that either water-soluble (nickel sulfate hexahydrate) or metallic nickel were carcinogenic. In addition, an oral carcinogenicity study resulting in high systemic absorption of nickel ions from nickel sulfate hexahydrate was negative for tumor induction (Heim et al., 2007). The findings of these animal studies indicate that water solubility alone is insufficient to explain the potential carcinogenicity of nickel compounds. Rather, the findings in rats suggest that other factors must influence the ability of inhaled nickel-containing substances to induce respiratory tumors. These factors may include the intrinsic respiratory toxicity of the substances, the lung clearance of nickel-containing particles, and the uptake of these particles by target epithelial cells in the respiratory tract and their intracellular dissolution, as described in the following sections. The evidence that these factors, rather than water solubility alone, influence the carcinogenic potential of nickel-containing substances supports the nickel ion bioavailability model.

4. Respiratory toxicity

The respiratory toxicity of inhaled nickel-containing substances critically affects the maximum exposure level of the substances that can be tolerated. In turn, and assuming that the particle size distribution of the aerosols are the same for all nickel substances, the tolerable exposure levels will affect the deposited doses in the lungs. This will influence the amount of nickel that is available for subsequent uptake and for delivery of the nickel ion to the nuclei of target cells. Thus, respiratory toxicity contributes to the ultimate nickel ion bioavailability and should be considered when assessing the carcinogenic potential of each form of nickel.

For nickel subsulfide and nickel oxide, the maximum exposure concentrations selected for the 2-year chronic studies were generally higher than those for nickel sulfate hexahydrate (Table 1) because these compounds are less toxic and, thus, have higher maximum tolerated doses (MTDs), as determined in earlier studies (Benson et al., 1986, 1989, 2002; Dunnick et al., 1988, 1989). The highest nickel sulfate hexahydrate exposure concentration in the 2-year chronic rat study was 0.11 mg Ni/m^3, and mild inflammatory lung lesions were observed just above this concentration in the 13-week studies (Dunnick et al., 1995; NTP, 1996a). These lung lesions were considered to be potentially life-threatening because of the possibility of reduced lung function. Benson et al. (2002)

also demonstrated a higher acute toxicity of nickel sulfate hexahydrate, finding significant mortality (31%) in rats exposed to 0.44 mg Ni/m^3 for one week and a high degree of pulmonary inflammation and cytotoxicity in surviving rats exposed to 0.22 mg Ni/m^3 for the remainder of the 13 weeks. In contrast, Benson et al. (2002) found that inhalation of 0.44 mg Ni/m^3 nickel subsulfide for 13 weeks resulted in only one death (2.6%). For nickel metal powder, high mortality rates were observed among rats exposed to 1 mg Ni/m^3 in the chronic study and the MTD was established at 0.4 mg Ni/m^3 (Oller et al., 2008). Based on these studies, the chronic toxicity of various nickel compounds in rats can be ranked as follows (in decreasing order): nickel sulfate hexahydrate (MTD = 0.11 mg Ni/m^3) > nickel metal powder (MTD = 0.4 mg Ni/m^3) > nickel subsulfide (MTD = 0.7 mg Ni/m^3) > nickel oxide (MTD = 2.0 mg Ni/m^3).

The toxicity of some, but not all, forms of nickel appears to be correlated with water solubility or solubility (i.e., nickel ion release) in the respiratory tract. Compared to nickel sulfate, nickel subsulfide released 16-fold less nickel, nickel metal powder released 310-fold less nickel, and nickel oxide was virtually insoluble, releasing 689-fold less nickel, in synthetic lung fluid after 24 hours (Oller et al., 2009). With the exception of nickel metal powder, which shows greater toxicity than nickel subsulfide, the nickel compounds with greater solubility in the respiratory tract are more toxic. These results suggest that one way in which the solubility of nickel compounds can affect bioavailability of nickel at target sites is by determining the overall toxicity of the exposure and, therefore, the overall maximum deposited nickel dose that can be achieved in test animals.

The greater toxicity of water-soluble nickel compounds, such as nickel sulfate hexahydrate, has been suggested to enhance the risks associated with exposure to carcinogenic agents. Cytotoxicity in the respiratory tract can trigger an inflammatory response, resulting in secondary effects such as oxidative damage to DNA or increased survival and proliferation of epithelial cells with precancerous changes through release of growth factors or other mitogens or through activation of signal transduction pathways (Goodman et al., 2009; Haber et al., 2000; Oller et al., 1997; Oller, 2002). Although it is possible that respiratory toxicity could play a role in carcinogenicity through one or more of these mechanisms, it is unlikely to play a large role because nickel sulfate hexahydrate was the most toxic nickel compound examined in rats, but was not carcinogenic (NTP, 1996a).

In summary, water-soluble nickel sulfate hexahydrate is the most toxic nickel compound in rats, followed by nickel metal powder, then nickel subsulfide and nickel oxide. Given that nickel subsulfide, which shows clear evidence of carcinogenicity in rats, is neither the most soluble nor the most toxic nickel compound, and that nickel sulfate hexahydrate, the most soluble and also the most toxic nickel compound, showed no evidence of carcinogenicity in rodents, the evidence suggests that carcinogenic potential is enhanced for nickel compounds with lower solubility and lower toxicity. Although particle size and toxicity are expected to limit inhalation exposures and, thus, lung deposition and body burden, it is the subsequent clearance that determines both the persistence of nickel in the lungs and the available dose for uptake by the target cells. Thus, the kinetics of nickel clearance from the lungs also affects the bioavailability of nickel-containing substances, as discussed below.

5. Clearance

Several studies have examined the lung clearance kinetics of inhaled or intratracheally-administered nickel-containing particles in animals. The retention half-time, or the time it takes for half of the lung burden to be cleared, and other relevant information from these studies are summarized in Table 2. These studies indicate that the form of nickel has a large effect on the lung clearance of inhaled or intratracheally delivered nickel, although lung clearance is only one of the many factors affecting carcinogenic potential.

A number of studies have observed the lung clearance kinetics of inhaled nickel as a biphasic process: a first, rapid phase followed by a prolonged slow phase (Valentine and Fisher, 1984; Finch et al., 1987; Benson et al., 1995a). One of the possible lung clearance pathways (contributing to the first, rapid component of the bimodal clearance curves) for nickel-containing particles that are only slightly soluble in biological media (such as sulfidic and oxidic nickel compounds) is the mechanical transport of phagocytized particles up the mucociliary escalator. The transport of nickel-containing particles via lung-associated lymph nodes or the dissolution to nickel ions of nickel-containing substances with some solubility may also contribute to lung clearance, although to a lesser extent (contributing to the second, slow component of the bimodal clearance curves) (Benson et al., 1995a).

Benson et al. (1994, 1995a, 1995b) conducted comparative lung clearance studies of nickel subsulfide, nickel oxide, and nickel sulfate hexahydrate. Benson et al. (1995a) continuously exposed rats to 0.62 or 2.5 mg nickel oxide/m^3 or to 0.12 or 0.5 mg nickel sulfate hexahydrate/m^3 for a period of 6 months. Clearance of inhaled nickel oxide was impaired in a dose-dependent manner, leading to accumulation of nickel in the lungs. In contrast, clearance was unaffected and nickel did not accumulate in the lungs after repeated inhalation of nickel sulfate hexahydrate, as more than 99% of lung-deposited nickel cleared with a half-time of less than 3 days, and the remaining nickel cleared over an indefinite period. In addition, exposure concentration and prior exposure to nickel sulfate hexahydrate had no significant effects on its clearance. In the case of nickel oxide, both exposure concentration and prior exposure to nickel oxide had significant effects on nickel clearance. Overall, clearance was considerably slower for nickel oxide than for nickel

Table 2. Clearance studies of nickel in animals.

Nickel compound	Species	Exposure route	Exposure duration	Retention half-time[a]	Reference
Sulfidic nickel					
Nickel subsulfide	F344/N rats	Pernasal inhalation	120 min	4.6 d	Benson et al. (1994)
β-Nickel subsulfide	F344/N rats	Inhalation	6h/d, 1–22 d	3.5–8 d	Benson et al. (1995c)
	Cynomolgus monkeys	Pernasal inhalation	45 min	4–5 d	Benson et al. (1995b)
Oxidic nickel					
Low-temperature (250°C) nickel oxide	Wistar rats	Intratracheal	Single administration	>90 d	English et al. (1981)
Nickel oxide (formed at 550°C)	Wistar rats	Inhalation		1 d (bronchial) and 36 d (alveolar)	Hochrainer et al. (1980)
High-temperature green nickel oxide	F344/N rats	Pernasal inhalation	120 min	120 d	Benson et al. (1994)
	Wistar rats	Inhalation	7h/d, 5d/wk, 12 mo	8–21 mo	Tanaka et al. (1995 and 1988)
High-temperature (1200°C) green nickel oxide	F344/N rats	Inhalation	6 h/d, 5 d/w; 6 mo	116–346 d	Benson et al. (1995a)
	Cynomolgus monkeys	Pernasal inhalation	45 min	>200 d	Benson et al. (1995b)
Water-soluble nickel					
Nickel chloride	Wistar rats	Intratracheal	Single administration	<3 d	English et al. (1981)
	Sprague-Dawley rats	Intratracheal	Single administration	<1 d	Carvalho and Ziemer (1982)
Nickel sulfate	F344/N rats	Intratracheal	Single administration	1–3 d	Medinsky et al. (1987)
Nickel sulfate hexahydrate	F344/N rats	Inhalation	6 h/d, 5 d/w; 6 mo	2.1 d (>99%)	Benson et al. (1995a)
	Cynomolgus monkeys	Pernasal inhalation	45 min	5 h (96%), then 10 d	Benson et al. (1995b)
Metallic nickel					
Nickel metal powder	Wistar rats	Inhalation	6 hr/d, 5d/wk, 90 d	26.5–110 d	WIL (2004)

[a]Two retention half-times are reported for studies looking at biphasic clearance kinetics, and the percent body burden associated with each phase is given in parentheses.

sulfate hexahydrate, with an estimated half-time of 116 to 346 days, depending on the exposure concentration. These results were consistent with those of Benson et al. (1994), who exposed rats through the nose to 9.9 mg nickel oxide/m^3 and 5.7 mg nickel subsulfide/m^3 for 70 and 120 minutes, respectively. The retention half-time of nickel oxide was 120 days, which is within the range observed in the Benson et al. (1995a) study. Clearance of nickel subsulfide was rapid, with a half-time of 4.6 days, but slower than that of nickel sulfate hexahydrate (as determined by Benson et al., 1995a).

The lung clearance of metallic nickel in animals is not well studied. WIL (2004) exposed rats to nickel metal powder at 1–8 mg Ni/m^3 for 6 hours per day, 5 days per week, for 90 days. Lung burdens were determined at 0, 30, 60, and 90 days after the exposure period. Depending on the exposure level, retention half-times ranged from 26.5 to 110 days. For the highest exposure level used in the carcinogenicity study with nickel metal powder (1.0 mg Ni/m^3), the retention half-time was 26.5 days in males and 53.3 days in females (G. Oberdörster, personal communication). The retention half-time at the MTD identified in the Oller et al. (2008) carcinogenicity study (0.4 mg Ni/m^3) was not measured in this 90-day study, but is assumed to be equal to or lower than the retention half-time at 1.0 mg Ni/m^3. This study suggests that the lung clearance of nickel metal powder is considerably slower compared to nickel sulfate hexahydrate and nickel subsulfide, but not as slow as that of nickel oxide.

Benson et al. (1995b) exposed monkeys via nasal inhalation to various nickel compounds to bridge the gap between rodent and human respiratory dynamics. Monkeys were exposed for 45 minutes to 15 mg nickel subsulfide/m^3, 25 mg nickel oxide/m^3, and 10 mg nickel sulfate hexahydrate/m^3. Nickel in the lungs of monkeys exposed to nickel subsulfide cleared with a half-time between 4 and 5 days. Clearance of nickel oxide appeared to be slower than in rats, with an estimated half-time greater than 200 days. Nickel sulfate hexahydrate was cleared rapidly from the lungs, with a retention half-time of approximately 5 hours for 95% of the nickel and 10 days for the remainder. This was faster than the retention half-time of 1 to 3 days observed in rats exposed to nickel sulfate hexahydrate by intratracheal instillation or inhalation (English et al., 1981; Carvalho and Zeimer, 1982; Medinsky et al., 1987; Benson et al., 1994, 1995a). Given the closer similarity of primates (versus rodents) to humans, the finding of faster clearance of nickel sulfate hexahydrate in monkeys than in rats suggests that clearance may also be faster in humans than in rats, at least for high acute exposures. The opposite may be true for the very insoluble nickel oxides, whereas similar clearance

rates may be expected for nickel subsulfide and perhaps metallic nickel. Definitive data on human lung clearance of nickel particles is lacking, however.

The effect of clearance on the bioavailability of inhaled nickel-containing particles is evident from the nickel lung burden observed in the NTP and several other studies. The lung burden reflects the rate of deposition and clearance of each nickel compound. In the NTP studies, the nickel lung burden in rats was measured after 15 months of exposure. For nickel subsulfide, the lung burden at 0.11 mg Ni/m^3 (the LOAEC for tumor induction) was 6 µg Ni/g control lung (NTP, 1996b). For nickel oxide, the lung burden at the LOAEC for tumor induction (1 mg Ni/m^3) was 1101 µg Ni/g control lung, and at the NOAEC (0.5 mg Ni/m^3) it was 310 µg Ni/g control lung (NTP, 1996c). For nickel sulfate hexahydrate, the lung burden at the MTD (0.11 mg Ni/m^3) was 2 µg Ni/g control lung (NTP, 1996a). Nickel lung burden for nickel metal powder at 15 months was extrapolated from values measured in rats exposed to 0.4 mg Ni/m^3 (the MTD) for 6 hours per day, 5 days per week for 12 months (Oller et al., 2008) and was determined to be 34 µg Ni/g control lung (A. Oller, personal communication). These nickel body burdens for sulfidic, oxidic, water-soluble, and metallic nickel are the result of deposited doses (limited by the MTD) and retention half-times.

In summary, the retention half-times vary remarkably for different nickel-containing substances, depending on solubility, exposure concentration and duration, and the species tested. In general, retention half-times in rats are 1 to 2 days for nickel sulfate hexahydrate, about 5 days for nickel subsulfide, between 30 and 50 days for nickel metal powder, and greater than 100 days for nickel oxide. Nickel lung burden, as a measure of retained dose, is a function of exposure, particle size, and clearance. For nickel sulfate hexahydrate, high toxicity and rapid clearance are two factors that lead to low retained doses and can explain the lack of carcinogenicity for this compound in animals. The retained dose alone cannot explain the differences in carcinogenicity between nickel subsulfide and nickel metal powder or between nickel subsulfide and nickel oxide as observed in the rat studies, however. Subsequent steps involving particle uptake and intracellular dissolution must also be considered, as discussed in the following sections.

6. Cellular uptake of nickel compounds

Although the extent of lung retention of nickel-containing particles does not completely explain the differing carcinogenic potentials of insoluble forms of nickel, the processes following lung deposition critically modify the intracellular, and ultimately intranuclear, bioavailability of the nickel ion. Cellular uptake of nickel-containing particles is a crucial factor, in the context of the nickel ion bioavailability model, that contributes to the observed differences in the carcinogenic potential of various nickel compounds. Cellular uptake of nickel-containing particles has been studied in vitro using various types of mammalian cells, including lung epithelial cells (as reviewed by Hack et al., 2007). These studies indicate that the cellular uptake of different forms of nickel is likely to occur primarily via two processes: ion-transport channels or endocytosis (which includes phagocytosis in macrophages). These two uptake processes and the factors that influence them are discussed below.

6.1. Cellular nickel uptake via ion-transport channels

The strongest evidence suggests that ion-transport channels are the primary route of nickel ion uptake into cells following the extracellular dissolution of water-soluble nickel compounds. Nickel ions from insoluble nickel compounds and metallic nickel can also be taken up into cells via this process, but extracellular dissolution of these substances is limited because of their lower predicted solubility in lung fluids, as described above (Oller et al., 2009).

The ionic radius of the nickel ion (0.66 Å) is similar to that of the magnesium ion (Mg^{2+}; 0.65 Å) and smaller than that of the calcium ion (Ca^{2+}; 0.99 Å); therefore, the nickel ion is able to pass through magnesium and calcium ion-transport channels to the extent permitted by competition with Mg^{2+}, Ca^{2+}, or other metal ions. Several mammalian cell lines have been used to identify calcium and magnesium ion-transport channels as a mechanism for cellular uptake of nickel from water-soluble nickel compounds, such as pneumocytes from adult rat, embryonic feline, and adult human lungs (Saito and Menzel, 1986); immortalized human kidney epithelial cells (Refsvik and Andreassen, 1995); isolated hepatocytes from male Wistar rats (Funakoshi et al., 1997); and V79 fibroblast cells (Hong et al., 1997). These studies have generally shown (1) decreased cellular nickel ion uptake in the presence of calcium ion-transport channel antagonists and (2) increased cellular nickel ion uptake in the presence of calcium ion-transport channel agonists and lower Ca^{2+} and/or Mg^{2+} concentrations.

Saito and Menzel (1986) reported up to 156% greater nickel ion accumulation from nickel chloride in various mammalian lung pneumocytes in Ca^{2+}-free medium compared to medium containing 2 mM calcium chloride (Saito and Menzel, 1986). Similarly, Funakoshi et al. (1997) reported a 19% increase in nickel ion uptake when male Wistar rat primary hepatocytes were treated with 10 µM nickel chloride in the absence of either Mg^{2+} or Ca^{2+}, and a 37% increase in the absence of both Mg^{2+} and Ca^{2+}. In another study, the presence of other metals—including zinc, cadmium, cobalt, and manganese—reduced nickel uptake from nickel chloride by human kidney epithelial cells by 90–95% (Refsvik and Andreassen, 1995). All of these observations suggested that cellular nickel ion uptake from water-soluble nickel chloride occurred through calcium or magnesium ion-transport channels and involved competition with other metal ions.

The specific involvement of calcium ion-transport channels in cellular nickel ion uptake from water-soluble nickel compounds was further demonstrated

by Refsvik and Andreassen (1995) and Funakoshi et al. (1997) using known calcium ion-transport channel antagonists and agonists. Nicardipine (a calcium channel antagonist) at 50 μM decreased nickel ion uptake (from 85 μM nickel) by 75% in human kidney epithelial cells, whereas ionomycin (a calcium channel agonist) at 3 mM increased nickel ion uptake 4- to 5-fold from the same amount of nickel (Refsvik and Andreassen, 1995). Pretreatment of cultures with the calcium channel antagonists nicardipine or verapamil decreased nickel ion uptake from 10 μM nickel chloride by 20%, whereas pretreatment with the calcium channel agonist vasopressin significantly increased nickel ion uptake from the same amount of nickel by 24% (Funakoshi et al., 1997). In addition, nickel ion uptake decreased by 20% at 4°C compared to 37°C, suggesting the involvement of carrier-mediated processes, which are temperature sensitive.

Abbracchio et al. (1982a) demonstrated that extracellular metal ion chelators, such as certain amino acids, can bind to nickel ions from water-soluble nickel compounds and prevent their cellular uptake. These chelators, as well as Ca^{2+} and Mg^{2+}, are generally present in vivo and can lower the cellular uptake of nickel ions. For example, Ca^{2+} and Mg^{2+} are present in synthetic alveolar lung fluid (which is representative of alveolar lung fluid in vivo) at concentrations of 100 and 24 μg ion/ml, respectively (as calculated from Stopford et al., 2003). These concentrations are much higher than the lung burden of 2 μg Ni/g lung determined for water-soluble nickel sulfate hexahydrate in the NTP (1996a) bioassay (assuming that 1 ml = 1 g, resulting in concentrations of 100 μg/g lung for Ca^{2+} and 24 μg/g lung for Mg^{2+}, respectively), suggesting that nickel ions from this compound would have to compete with much higher levels of Ca^{2+} and Mg^{2+} for uptake into cells. Extracellular nickel ion concentrations have to be high enough relative to those of other cations and extracellular chelators for entry into cells. Given the low retained dose of water-soluble nickel that can be achieved in vivo (as described above), the ultimate result of the interaction of these two factors (low retained dose and low intracellular uptake) is predicted to be very low nuclear bioavailability of nickel ions from water-soluble nickel compounds. This is generally not the case for insoluble nickel compounds, which have limited extracellular dissolution and are present in the respiratory tract as particles that are primarily taken up via endocytosis. Because water-soluble nickel compounds dissolve readily in extracellular fluids, they are not expected to be present as particles, and this is supported by animal studies in which no particles were observed in the lung tissues of rats exposed to nickel sulfate hexahydrate (NTP 1996a; Benson et al., 1995a). It is unclear how the low intracellular uptake of nickel ions from water-soluble nickel compounds can result in their relatively rapid lung clearance via urinary excretion (Hack et al., 2007).

6.2. Cellular nickel uptake via endocytosis

A number of studies using various cell types have demonstrated that endocytosis is the primary mechanism of in vitro cellular nickel ion uptake from insoluble nickel compounds. Benson et al. (1992) have demonstrated that lung epithelial cells, the target cells for nickel carcinogenicity, are capable of endocytosing nickel subsulfide particles in vitro, suggesting that endocytosis could be a biologically relevant route in vivo, as well. The efficiency of nickel endocytosis is influenced by the chemical form of nickel and the physical structure (i.e., crystallinity), surface charge, shape, and size of the nickel-containing particles.

The influence of the chemical form of nickel compounds on their cellular uptake is apparent in many studies. Briefly, nickel monoxides (NiO), referred to here as nickel oxides, include a spectrum of compounds from a high-temperature green variety to low-temperature black products. High-temperature green nickel oxide is relatively inert and is the predominant form in nickel refineries, whereas black nickel oxides are more chemically active. Complex nickel oxides, such as copper-nickel oxides, are often formed as by-products of industrial processes. Copper-nickel oxide is more reactive than black nickel oxides. Sulfidic nickel generally consists of nickel sulfide compounds, such as nickel sulfide (NiS) and nickel subsulfide (Ni_3S_2), that occur as intermediates in the processing of sulfidic ores. Nickel subsulfide exists in two forms: the low-temperature green form, α-Ni_3S_2 (also known as the mineral heazlewoodite), and the high-temperature bronze-yellow form (β-Ni_3S_2). Nickel sulfide exists as one of three compounds (α-NiS, β-NiS, or amorphous NiS) in the form of dark green to black crystals or powder. Metallic nickel and nickel oxides are taken up via endocytosis to a lesser extent than sulfidic nickel particles, and water-soluble nickel does not appear to be endocytosed to any great extent, if at all. Sunderman et al. (1987) reported endocytotic indices for ten different nickel oxides and nickel-copper oxides in C3H-10T1/2 cells that were in the range of less than 1% to 7.2% (with indices for nickel-copper oxides being generally greater than those for nickel oxides) and that were all less than the index for crystalline β-nickel sulfide (β-NiS). Costa et al. (1981a) exposed Chinese hamster ovary (CHO) cells to similar concentrations of metallic nickel, nickel oxide, crystalline nickel subsulfide (α-Ni_3S_2), and crystalline α-nickel sulfide (α-NiS) and found indices of 0–1.2% for metallic nickel and nickel oxide and of 6.1–23% for the sulfidic nickel compounds. Costa and Heck (1982) reported indices of 22–27% for crystalline α-nickel sulfide, 2–5% for nickel oxides, 4% for metallic nickel, and 0% (non-detect) for water-soluble nickel chloride in CHO cells. Other studies have also demonstrated that sulfidic forms of nickel, particularly in crystalline forms, appear to be more readily taken up via endocytosis than other nickel compounds, such as amorphous nickel sulfide (NiS), metallic nickel, and nickel oxides (Abbracchio et al., 1981, 1982b; Costa and

Mollenhauer, 1980a, 1980b; Costa et al., 1981b; Miura et al., 1989).

The physical structure of nickel-containing particles (i.e., whether they are crystalline or amorphous) has a large impact on the endocytosis of nickel compounds (Costa and Mollenhauer, 1980a, 1980b; Costa et al., 1981a; Abbracchio et al., 1981, 1982b; Miura et al., 1989). Costa and Mollenhauer (1980a,1980b) exposed Syrian hamster embryo (SHE) and CHO cells to both crystalline nickel subsulfide and amorphous nickel sulfide. Both types of nickel-containing particles were of similar size (≤5 μm), yet both cell lines actively endocytosed the crystalline particles but did not take up the amorphous particles. For example, within 30 minutes after addition of crystalline nickel subsulfide (at high dose) to cell cultures, 12.5% of the cells contained nickel particles in the cytoplasm; within 6 hours, 75% of the cells had engulfed the particles. In contrast, less than 1% of the cells exposed to amorphous nickel sulfide contained particulate nickel within the 6-hour post-exposure period. At 0.1 to 10 μg/ml, crystalline nickel subsulfide had endocytotic indices of 0.8–42.9% in SHE cells and 2.2–79.3% in CHO cells, and amorphous nickel sulfide had endocytotic indices of 0–0.81% in SHE cells and 0–3.5% in CHO cells (Costa and Mollenhauer, 1980b). Abbracchio et al. (1981) reported similar results using several cell lines, including secondary SHE cells, CHO cells, baby hamster kidney (BHK-21 C-13) cells, WI-38 human diploid fibroblasts, and VA-13-transformed WI-38 fibroblasts. Cells were incubated with amorphous nickel sulfide, crystalline α-nickel sulfide, or crystalline nickel subsulfide particles. In the animal cells (secondary SHE, CHO, and BHK-21), the endocytotic indices were about 1%, 13–21%, and 24–30% for amorphous nickel sulfide, crystalline α-nickel sulfide, and crystalline nickel subsulfide, respectively. In the human cells, however, endocytotic indices were non-detect (ND) for crystalline α-nickel sulfide, ND to 1.6% for amorphous nickel sulfide, and ND to 14.3% for crystalline nickel subsulfide, indicating the possibility of species or cell-type specificity in vitro.

The influence of the size and shape of nickel-containing particles on their potential for endocytosis was shown in a study by Miura et al. (1989), in which 10T1/2 mouse embryo cells were exposed to 1–50 μM of insoluble nickel compounds (crystalline nickel subsulfide, crystalline α-nickel sulfide, and nickel oxide) for 48 hours. In this study, crystalline nickel subsulfide particles were heterogeneous in size, crystalline α-nickel sulfide particles were smaller and more homogeneous in size, and nickel oxide particles were very fine. The range of particle sizes for each specific compound was not reported, but all particles were less than 5 μm in diameter. Both the crystalline nickel subsulfide and crystalline α-nickel sulfide particles disappeared from the culture within 1 week, but the crystalline nickel subsulfide particles were endocytosed only moderately well and not as readily as the smaller crystalline α-nickel sulfide particles. In contrast, the very fine nickel oxide particles showed only minimal endocytotic uptake and persisted in culture for 6 weeks; these particles appeared to attach to the cell surface and did not concentrate in cytoplasmic vacuoles. A direct influence of particle size on endocytosis was demonstrated by Costa et al. (1981a) using crystalline α-nickel sulfide particles of varying sizes. Particles less than 4 μm in diameter were actively endocytosed by CHO and BHK-21 cells, with at least 30% of cells containing endocytosed nickel particles. The use of particles ranging in size from 4 to 5 μm reduced endocytosis substantially, with only up to 15% of cells showing active endocytosis of 5-μm particles. The cell lines did not actively endocytose particles greater than 5 μm, and amorphous nickel sulfide was not actively endocytosed, regardless of particle size. Particle sizes much greater than 1 μm in diameter would not be relevant to the animal inhalation studies conducted by NTP (1996a, 1996b, 1996c) and Oller et al. (2008), as the MMAD for the aerosols used in those studies ranged from 1.8 to 3 μm.

Another factor that influences the potential for endocytosis of nickel-containing particles is the surface charge of the particles, with negatively-charged particles being more readily endocytosed than positively-charged particles (Abbracchio et al., 1981, 1982b; Heck and Costa, 1982; Costa, 1983). Abbracchio et al. (1981, 1982b) exposed CHO cells to the same concentrations of crystalline and amorphous nickel sulfide particles before and after surface reduction with lithium aluminum hydride ($LiAlH_4$), a process that produces a more negatively-charged surface. Following surface reduction, endocytotic indices increased from 33.3% to 51.0% for crystalline α-nickel sulfide and from 4.0% to 9.3% for amorphous nickel sulfide. Upon investigating the surface properties, Abbracchio et al. (1982b) found that the crystalline α-nickel sulfide particles had negatively-charged surfaces whereas the amorphous nickel sulfide particles had positively-charged surfaces. Subsequent X-ray photoelectron spectroscopic (XPS) analysis of the particles revealed striking differences in the nickel/sulfur ratios and the sulfur oxidation states of the outermost surface (1–4 nm) of the two types of particles. Reduction of particle surfaces with $LiAlH_4$ enhanced their endocytosis, and in the case of amorphous nickel sulfide, the surface reduction resulted in an incidence of morphological transformation of SHE cells comparable to that observed with untreated crystalline α-nickel sulfide. Similar findings were reported by Heck and Costa (1982), who found that uptake of crystalline and amorphous nickel sulfide by mammalian cells increased from 29% to 53% and from 5% to 28%, respectively, following surface reduction with $LiAlH_4$.

Other factors besides the inherent physicochemical characteristics of nickel-containing particles that may modify cellular nickel uptake have been studied, but not extensively. For example, nickel metal and most of its alloys are known to passivate, which involves the formation of a non-equilibrium film that effectively blocks oxidizing agents from reacting with the metal to form divalent nickel ions (Revie and Uhlig, 2008). The

passivating films are extremely thin (on the order of monolayers) and are not detectable by electron diffraction. Passivating films decrease the surface reactivity of various types of metal particles and may contribute to the poor intracellular uptake of nickel metal particles (and metallic forms of many other metals) observed in in vitro studies (Costa et al., 1981b). In addition, co-exposures to other substances could affect uptake. Costa et al. (1981a) suggest that manganese (Mn) dust co-incubated with crystalline nickel subsulfide in CHO cells inhibits the induction of morphological transformation by this nickel compound, and it is presumed that the process of cellular transformation by a nickel compound requires uptake into the cell. In addition, Mn dust alone was not endocytosed, but either Mn dust or Mn^{2+} ions inhibited the endocytosis of crystalline α-nickel sulfide, indicating that the inhibition takes place extracellularly. In the same study, the presence of amorphous nickel sulfide also inhibited the endocytosis of crystalline α-nickel sulfide. These results suggest that the co-exposure of a mammalian cell line to another nickel compound or other metal compounds may affect the cellular uptake of a specific nickel compound via endocytosis, and this modulation of cellular uptake may also occur in vivo when exposures are complex, such as in workplaces that process and refine nickel.

6.3. Relative cellular uptake of various nickel compounds

The complexity of nickel uptake processes is not completely understood. The cellular uptake of nickel ions from water-soluble nickel compounds via ion-transport channels does not appear to be as efficient for delivery of nickel ions to nuclear sites as uptake of nickel-containing particles via endocytosis (Harnett et al., 1982; Hack et al., 2007). For example, only 0.18% of total nickel was taken up by CHO cells when exposed to 1 mg water-soluble nickel chloride compared to 14.7% when cells were exposed to 1 mg water-insoluble crystalline nickel sulfide under similar conditions (Harnett et al., 1982). Metallic nickel, nickel oxides, and amorphous nickel sulfide do not undergo sufficient extracellular dissolution to be taken up via ion-transport channels, and although they are able to be taken up into cells via endocytosis, they are not as readily taken up by this mechanism as crystalline nickel subsulfide (Kuehn et al., 1982; Miura et al., 1989). Available in vitro studies suggest that the relative extent of cellular uptake of various nickel-containing substances is likely to follow the general trend (in increasing order): water-soluble nickel < metallic nickel < amorphous nickel sulfide < [nickel oxide < nickel-copper oxides] < crystalline nickel sulfide < crystalline nickel subsulfide. Consequently, the potential for nickel ions from water-soluble nickel compounds, nickel monoxides, metallic nickel, and amorphous nickel sulfide to accumulate in the nucleus and interact with DNA is much lower than that of crystalline nickel subsulfide, which is readily taken up by mammalian cells

7. Dissolution and intracellular fate of nickel species

Once nickel particles or nickel ions are taken up by target cells, they undergo dissolution processes through which some nickel ions may be ultimately transported to the nucleus. Below, the intracellular fate of endocytosed nickel-containing particles and ion channel-transported nickel ions is discussed.

In vitro studies in mammalian cell lines indicate that endocytosed nickel-containing particles are initially retained within cytoplasmic vacuoles, where they undergo slow dissolution (Abbracchio et al., 1981, 1982a; Costa et al., 1981b; Costa and Mollenhauer, 1980a, 1980b; Evans et al., 1982; Hildebrand et al., 1990, 1991). The cytoplasmic vacuoles move from the periphery of the cells to perinuclear areas, but the nickel-containing particles remain within the vacuoles and have not been observed in the nucleus (Evans et al., 1982). In addition, the nucleus is relatively impermeable toward larger nickel-containing particles (e.g., to crystalline nickel sulfide particles greater than 0.1 μm; Costa et al., 1981a). For nickel ions to interact with nuclear DNA and potentially elicit carcinogenic effects, the endocytosed nickel particles must dissolve within the vacuoles and the resulting nickel ions must be transferred to the nucleus (Hildebrand et al., 1990).

Dissolution of nickel-containing particles inside the vacuoles to release nickel ions was demonstrated in several studies of sulfidic nickel (Abbracchio et al., 1982c; Costa et al., 1982; Evans et al., 1982) and the interaction of the vacuoles with lysosomes and their possible fusion has also been reported (Costa et al., 1982; Evans et al., 1982). The interaction between vacuoles and lysosomes exposes the endocytosed nickel-containing particles to the acidic content of the lysosome, which may enhance the dissolution rate of some nickel-containing particles. Endocytosis of amorphous nickel sulfide particles can also increase lysosomal activity, increasing the potential for interaction between lysosomes and the cytoplasmic vacuoles and further increasing the dissolution rate of nickel-containing particles (Abbracchio et al., 1982b).

Transport of nickel ions from cytoplasmic vacuoles into the nucleus may occur via diffusion across the nuclear membrane or via merging of the vacuoles to the nuclear membrane, resulting in direct delivery of nickel ions to the nucleus. The merging of vacuoles is less likely, however, as it would result in undissolved particles being present in the nucleus, and this has not been observed. If particles in the vacuoles are resistant to intracellular dissolution, the nickel ion dose would be lower, resulting in a decreased carcinogenic effect. For example, nickel oxide particles that are endocytosed are not well-solubilized in the cytoplasm (Miura et al., 1989), and this could contribute to nickel oxide's lower carcinogenicity compared to nickel subsulfide (NTP, 1996b, 1996c). Data regarding the intracellular dissolution of metallic nickel particles are not available. Nickel metal does not react rapidly

with diluted, non-oxidizing acids or organic acids, but increased nickel ion release over time is expected under oxidizing conditions with low pH (Oller et al., 2009). Given the poor endocytotic uptake of metallic nickel particles and their relatively slow intracellular dissolution, based on corrosion via oxidation, the yield of intracellular nickel ions from metallic nickel particles is expected to be low (Sivulka, 2005; Oller et al., 2009).

Water-soluble nickel salts (e.g., nickel chloride) in their ionic form have the capacity to enter cells and nuclei. As discussed above, extracellular amino acids and proteins will bind nickel ions, and other divalent cations will compete with the remaining free nickel ions for cellular uptake. The majority of free nickel ions that do enter the cell are expected to bind to intracellular ligands, such as proteins, increasing cytotoxicity and severely limiting the amount of nickel ions that can enter the nucleus (Haber et al., 2000). Abbracchio et al. (1982a) demonstrated a 14-fold decrease in uptake of nickel ions into cell lines when the cell medium contained physiological levels of amino acids and proteins compared to a minimal medium containing only glucose and salts. In contrast, the nickel ions released from endocytosed insoluble nickel particles within cytoplasmic vacuoles are "protected" from interactions with intracellular ligands and are not expected to increase cytotoxicity, so these nickel ions may reach the nucleus more easily, particularly if they are released near it (Haber et al., 2000).

The intracellular dosimetry model of inhaled nickel compounds developed by Hack et al. (2007) predicted that endocytosis of nickel-containing particles is more effective for nuclear delivery of nickel ions than cellular uptake via ion channels. This model predicted that a 1 μM dose of nickel subsulfide resulted in a nuclear nickel ion concentration in human lung A549 cells that was 100-fold higher than that from a 1 μM dose of water-soluble nickel chloride. This has also been demonstrated in several in vitro studies. Abbracchio et al. (1982c) reported that nickel retention by the nuclei of intact cells was higher (30% of nickel) than that by isolated nuclei (10% of nickel) when both were exposed to amorphous nickel sulfide particles. Costa et al. (2005) demonstrated that exposure of A549 cells to nickel sulfide particles resulted in the majority of nickel ions being localized in the nucleus, whereas exposure to nickel chloride resulted in most of the ions being localized in the cytoplasm. Recent results from Ke et al. (2007) showed that nickel ions persisted in the cytoplasm and nuclei of human lung cells for a longer period following exposure to 1 μg/cm^2 nickel subsulfide than following exposure to 1 mM nickel chloride, suggesting that uptake by endocytosis results in a greater interaction of nickel ions with the nucleus than uptake via ion-transport channels. This is supported by a study in which the ratio of nickel content in the nucleus vs. in the cytoplasm of human lung cells was found to be higher after treatment with 0.2–2.0 μg/cm^2 black nickel oxide (>0.50) compared to treatment with 100–500 μM nickel chloride (0.10–0.18; Schwerdtle and Hartwig, 2006).

Evidence for increased delivery of nickel ions to the nucleus from endocytosed insoluble nickel particles is also supported by studies of the effects of nickel compounds on DNA. Damage to heterochromatin was observed in CHO cells after exposure to crystalline nickel sulfide, but not after exposure to water-soluble nickel chloride (Sen and Costa, 1985, 1986). When nickel chloride was packaged in liposomes and subject to endocytosis, however, the cellular uptake of nickel ions was much higher than with nickel chloride in solution and damage to heterochromatin was observed (Sen and Costa, 1986). This damage was greater with nickel chloride–bovine serum albumin (BSA) complexes, presumably because the large BSA molecule allows nickel ions to be retained in a phagocytized vacuole inside the cell. Other in vitro studies also suggest that exposure to insoluble nickel particles that are actively endocytosed may lead to direct or indirect interactions of nickel ions with DNA, resulting in neoplastic transformation (Abbracchio et al., 1982b; Costa et al., 1979, 1981a; Landolph, 1994; Miura et al., 1989; also reviewed by Costa et al., 2005). The insoluble compounds nickel subsulfide, crystalline nickel sulfide, and nickel oxide strongly induced morphological transformation of mouse embryo fibroblasts (Miura et al., 1989) with a potency that has been shown to correlate with their phagocytic uptake (correlation coefficient = 0.97; J. Landolph, personal communication). In contrast, Miura et al. (1989) demonstrated that soluble nickel sulfate and nickel chloride did not induce morphological transformation of mouse embryo fibroblasts. Thus, it appears that dissolution from endocytosed insoluble nickel particles in the vacuoles may lead to increased bioavailability of nickel ions in the nucleus compared to that which can be achieved from equivalent exposures to water-soluble nickel compounds.

8. Predictions from the nickel ion bioavailability model

After exposure to a nickel-containing substance, the nickel ion bioavailability model predicts the likelihood that nickel ions will be present in the nucleus of target lung epithelial cells in sufficient amounts to initiate tumorigenesis. The predictions of the nickel ion bioavailability model are based on the interactions of the various factors described above (Figure 1). The particle size of the aerosol and the intrinsic respiratory toxicity of a nickel-containing substance will limit the exposure level that can be achieved, whereas the combination of particle size, exposure level, and exposure duration will determine the maximum deposited dose in various regions of the respiratory tract. Particle clearance will affect the ultimate retained dose of a nickel-containing substance in the lungs. Intracellular uptake, and both the extracellular and intracellular dissolution, of a nickel-containing substance will further contribute to the ultimate bioavailability of nickel ions at nuclear sites in lung epithelial cells.

The nickel ion bioavailability model predicts that when the highest achievable nickel ion bioavailability at nuclear sites is above the threshold for tumorigenic effects, increased tumor incidence rates will be observed in animal studies and a carcinogenic hazard will be identified. When the highest achievable nickel ion bioavailability at nuclear sites is below the threshold for tumorigenic effects, no increased tumor incidence rate will be observed in animal studies and no carcinogenic hazard will be identified. In addition to the bioavailability of nickel ions at nuclear target sites, the carcinogenic potential of nickel-containing substances also depends on the probability that the presence of nickel ions in the nucleus can lead to a mutation in a proto-oncogene or to the inactivation of a tumor suppressor gene, as well as the probability of the cell surviving in the presence of nickel cytotoxicity or other causes of cell death before a tumor can be initiated.

Because nickel ions are only weakly genotoxic, other mechanisms for nickel carcinogenicity have been proposed. Some of these mechanisms do not involve the presence of nickel ions in the nucleus. One such mechanism is through interference of iron homeostasis, leading to activation of signal transduction pathways and alterations in gene expression that can promote the survival and proliferation of cells with precancerous changes (reviewed by Costa et al., 2005). Another, as discussed above, is through inflammatory responses triggered by the cytotoxic effects of, for example, water-soluble nickel compounds, leading to increased proliferation of cells that may have been initiated by a carcinogenic agent (Oller et al., 1997; Oller, 2002). These mechanisms describe potential tumor-promoting effects of nickel that only require nickel ions to be present at the cell membrane. Although these mechanisms cannot be dismissed as contributors to the enhancement of cancer risks when exposures to tumor initiators are also present, they are not likely to lead to tumor formation in the absence of other exposures. This is demonstrated by the lack of carcinogenicity of nickel sulfate hexahydrate in rats exposed orally (Heim et al., 2007) and by inhalation (NTP, 1996a) at exposure levels that (1) caused respiratory toxicity and (2) would have resulted in maximum interaction of nickel ions with cell membranes.

Other non-genotoxic mechanisms for nickel carcinogenesis that likely require nickel ions in the nucleus have been proposed. These include damage to heterochromatin, as discussed above, as well as DNA damage via production of reactive oxygen species (ROS); alteration of gene expression through DNA hypermethylation or effects on histones; and inhibition of DNA repair processes (reviewed by Beyersmann and Hartwig, 2008; Costa et al., 2005; Goodman et al., 2009). All of these mechanisms involve an indirect interaction of DNA with nuclear-available nickel ions, so the potential of nickel compounds to elicit any of these effects in vivo depends on their ability to deliver a sufficient amount of nickel ions to the nucleus.

Sulfidic forms of nickel, such as nickel subsulfide, have an intermediate toxicity and lung clearance rate, but are readily taken up into mammalian cells by endocytosis. Following endocytosis, nickel subsulfide particles are retained within cells via processes that tend to enhance their dissolution and nuclear delivery of nickel ions. Thus, newly released nickel ions from nickel subsulfide have a high predicted nuclear bioavailability. This correlates with the results of animal studies, in which nickel subsulfide increased tumor incidence in rats at levels ≥0.11 mg Ni/m^3 (NTP, 1996b), equivalent to 0.36–2.32 mg Ni/m^3 of workplace exposure. Human equivalent concentrations were calculated as described by Oller and Oberdörster (2010), using information on particle size distribution from the NTP studies and Oller et al. (2008) for animal exposure and from Yu et al. (2001) for human exposure (see Tables 5 and 6).

Copper-free nickel oxides have lower toxicity and are cleared very slowly from the lungs, resulting in relatively high lung retention. Yet, nickel oxides neither undergo sufficient extracellular dissolution to be taken up into cells via ion-transport channels nor are they readily taken up via endocytosis. They also undergo very slow intracellular dissolution. The potential for the delivery of nickel ions to the nucleus will be the result of the large retained dose and less-efficient uptake and intracellular dissolution. This potential is expected to be somewhat low, but sufficient for direct tumor induction in rats. In the case of high-temperature green nickel oxide, tumors were observed at exposure levels ≥1.0 mg Ni/m^3 under conditions of impaired particle clearance (NTP, 1996c). This exposure level is equivalent to 3.5–22 mg Ni/m^3 of workplace exposure. Thus, a 10-fold higher nickel oxide exposure compared to nickel subsulfide was required to observe tumors in rats. Other nickel oxides that may have higher rates of intracellular dissolution (e.g., nickel-copper oxides) may have carcinogenic potencies that are closer to that of nickel subsulfide. For example, Sunderman et al. (1990) observed an increased incidence of tumors in rats exposed to nickel-copper oxides compared to those exposed to an equivalent concentration of nickel monoxides via intramuscular injection. Although this study does not address inhalation exposures, it supports a greater carcinogenic potential for nickel-copper oxides.

It is unlikely that water-soluble nickel compounds, such as nickel sulfate hexahydrate and nickel chloride, can provide a sufficient amount of nickel ions to the nucleus of respiratory epithelial cells to initiate carcinogenicity. The high toxicity, rapid lung clearance, and uptake processes that deliver nickel ions to the cytoplasm where they can bind to intracellular ligands all lead to low nickel ion bioavailability in the nucleus, thus decreasing their carcinogenic potential (Figure 1). This is consistent with the lack of lung tumors observed with water-soluble nickel compounds in animal studies at exposure levels of ≤0.11 mg Ni/m^3 (NTP, 1996a), equivalent to 0.38–0.95 mg Ni/m^3 of workplace exposure. This lack of evidence for

carcinogenicity is likely not attributable to rats being an inappropriate species for evaluating the carcinogenicity of water-soluble nickel. Cobalt sulfate heptahydrate induced respiratory tumors in rats after inhalation at the same metal exposure levels used for nickel sulfate hexahydrate (NTP, 1998), providing evidence that the rat is a sensitive model for studying the inhalation carcinogenicity of water-soluble metal salts.

Metallic nickel has a relatively high toxicity and an intermediate retention half-time. It is not readily taken up by cells, but the release of nickel ions from metallic nickel particles can be increased under acidic conditions. Based on the current data, it is difficult to predict the overall nuclear bioavailability of nickel ions from metallic nickel. No respiratory tumors were observed in rats after inhalation exposure to ≤0.4 mg Ni/m^3 nickel metal powder (Oller et al., 2008), equivalent to 1.2–8.5 mg Ni/m^3 of workplace exposure, indicating that the overall nickel ion bioavailability must have been below the threshold for tumor induction. Given that the lung burden of nickel metal powder at the MTD was 6-fold higher than the minimal lung burden that resulted in tumors with nickel subsulfide (Oller et al., 2008; NTP, 1996b), the poor cellular uptake and intracellular dissolution of metallic nickel appear to be the driving factors for its lack of carcinogenicity.

In contrast to the original nickel ion theory, from which one can extrapolate the highest risks from water-soluble nickel, intermediate risks from sulfidic and metallic nickel, and low or no risk from oxidic nickel, the results of chronic inhalation studies in animals indicate clear evidence of respiratory carcinogenicity in rats for nickel subsulfide, some evidence for nickel oxide, and no evidence for nickel sulfate hexahydrate and nickel metal powder (NTP, 1996a, 1996b, 1996c; Oller et al., 2008). These findings are supported by the oral carcinogenicity study of nickel sulfate hexahydrate in rats conducted by Heim et al. (2007). No treatment-related increase in tumor incidence was observed in this study, even though oral exposures of water-soluble nickel lead to the highest potential internal exposure to nickel ions (Ishimatsu et al., 1995; Hayman et al., 1984). Because there were no systemic tumors detected, and epidemiological studies have not consistently shown cancers at sites other than the respiratory tract (ICNCM, 1990), it is not likely that the presence of nickel ion alone determines carcinogenicity. Rather, it is whether the nickel ion can reach the nucleus of lung epithelial cells that will determine carcinogenicity. This process is best described by the nickel ion bioavailability model, which predicts an increased incidence of lung cancer in humans exposed to sulfidic and certain oxidic nickel compounds, but no increased risks after exposure to just water-soluble nickel compounds or metallic nickel.

9. Epidemiology studies

Based on the nickel ion theory, one could extrapolate an increased incidence of lung cancer in workers exposed to sufficiently high levels of any form of nickel, with risks proportional to the solubility of nickel. That is, the potency of nickel substances is predicted to follow the extent of nickel ion release in water: water-soluble nickel compounds >> sulfidic nickel compounds = metallic nickel > oxidic nickel compounds. The nickel ion bioavailability model, on the other hand, predicts an increased incidence of lung cancer in workers exposed to high levels of sulfidic and certain oxidic nickel compounds; with no increased risks in workers exposed to water-soluble nickel compounds or metallic nickel alone.

Over the last century, dozens of occupational epidemiological studies have assessed respiratory cancer risks associated with nickel exposures. Although a causal role for nickel exposures from the refining and processing of sulfidic mattes was evident by the 1950s (Doll, 1958; Løken, 1950; Sutherland, 1959), results for specific forms of nickel are less clear. Workers in different industries, and those with different duties within the same industry, were often exposed to different combinations of several forms of nickel and other non-nickel substances, and had other dissimilarities (e.g., smoking habits, other lifestyle factors), making it challenging to determine which specific forms are correlated with increased risk. (These and other factors that likely affected interpretation of epidemiological results are described in detail by Goodman et al. [2009]).

In the 1980s, the International Committee on Nickel Carcinogenesis in Man (ICNCM) initiated the first comprehensive study aimed at assessing respiratory cancer risks of specific nickel forms among 10 cohorts of 80,000 people working predominantly in the primary production of nickel and the processing of nickel alloys. Five of these cohorts were made up of workers who refined and processed sulfidic nickel ores at the Mond/INCO operation in Clydach, Wales; the Falconbridge refinery in Kristiansand, Norway; the INCO refining/sintering operations in Port Colborne and Copper Cliff, Ontario, Canada; the INCO Huntington Alloys plant (which had a calcining department that was operational until 1947) in West Virginia, USA; and the Outokumpu Oy smelter and refinery in Finland. These workers were exposed to varying amounts of sulfidic, oxidic, water-soluble, and metallic nickel (Table 3). Two cohorts exposed to low levels of sulfidic, oxidic, and water-soluble nickel were the non-sinter workers (engaged in mining, milling, smelting, iron ore recovery, copper refinery jobs) at INCO's Sudbury operations and workers at the Falconbridge mining and smelting operations in Ontario, Canada. Workers at the Societé le Nickel mining and smelting operations in New Caledonia and the Hanna mining and smelting operations in Oregon, USA, two cohorts who worked with lateritic ore, were exposed primarily to oxidic nickel and almost no sulfidic or water-soluble nickel. Workers at the Henry Wiggin Alloy Company in Hereford, England, were exposed to low levels of oxidic and metallic nickel. The final cohort was made up of workers at the Oak Ridge Gaseous Diffusion Plant, which produced barrier

Table 3. Nickel industry exposures and rat bioassay nickel human equivalent concentrations (HECs).

Industry sector/cohort[a]	Estimated exposures (mg Ni/m³)				Soluble nickel equivalents		Reference
	Sulfidic	Oxidic	Soluble	Metallic	Alveolar	Interstitial	
Refining operations with high insoluble and water-soluble nickel exposures							
Linear Calcining Department, Mond/INCO Refinery, Clydach, Wales	6.75 – 9	16.5 – 18.75	0.75	3 – 5.25	0.89	2.24	ICNCM (1990)
Copper Plant, Mond/INCO Refinery, Clydach, Wales	0.01 – 0.38	0.4 – 13	0.01 – 1.13	0	0.62	0.67	ICNCM (1990)
Sinter Plant, Copper Cliff, Ontario, Canada	3 – 35	5 – 60	<4	0	3.05	6.31	ICNCM (1990)
Leaching, Calcining, and Sintering, Port Colborne, Ontario, Canada	2 – 20	3 – 40	<3.0	0	2.12	4.01	ICNCM (1990)
Roasting, Smelting, and Calcining, Falconbridge Nickel Refinery, Kristiansand, Norway [b]	0 – 2	0 – 8	0	0 – 2	0.08	0.25	ICNCM (1990)
Roasting, Smelting, and Calcining, Falconbridge Nickel Refinery, Kristiansand, Norway [c]	0.02 – 0.8	0.3 – 3.8	0.06 – 0.53	0 – 0.16	0.32	0.40	Grimsrud et al. (2000)
Calcining, Huntington Alloys, Inc., West Virginia, USA (before 1947)	0 – 4	0 – 0.5	0 – 0.05	0 – 0.4	0.13	0.46	ICNCM (1990)
Refining operations with low insoluble and high water-soluble nickel exposures							
Electrolysis, Falconbridge Nickel Refinery, Kristiansand, Norway [b]	0 – 2	0 – 2	<0.5 – 8	0 – 2	4.32	4.49	ICNCM (1990)
Electrolysis, Falconbridge Nickel Refinery, Kristiansand, Norway [c]	0.001 – 0.01	0.003 – 0.016	0.025 – 0.17	0.001 – 0.002	0.10	0.10	Grimsrud et al. (2000)
Hydrometallurgy, Mond/INCO Refinery, Clydach, Wales	0.05	0.9	1.3	0.5	1.3	1.3	ICNCM (1990)
Electrolysis, Port Colborne, Ontario, Canada	<0.8	<0.2	<0.3 – 3	<0.5	1.67	1.74	ICNCM (1990)
Outokumpu Oy Refinery, Harjavalta, Finland	0.06 – 0.4	0	0.25	0	0.26	0.30	Anttila et al. (1998)
Outokumpu Oy Smelter, Harjavalta, Finland	0.02 – 0.2	0	0	0	0.01	0.02	Anttila et al. (1998)
Refining operations with high metallic nickel exposures							
Hydrometallurgy, Saskatchewan, Alberta, Canada	0	0	0	2.0 – 4.0	0.05	0.04	Egedahl et al. (2001)
Mining and smelting of sulfidic ores with low insoluble and water-soluble nickel exposures							
Mining, Milling, Smelting Operations, Falconbridge, Ontario, Canada	0.02 – 0.2	0 – 0.02	0 – 0.01	0	0.01	0.03	ICNCM (1990)
Mining, Milling, Smelting Operations, INCO, Ontario Canada	<0.5	<1	<0.2	<0.1	0.12	0.16	ICNCM (1990)
Mining and smelting of lateritic ores							
Societe le Nickel Mining and Smelting Operations, New Caledonia	≤ 0.1	<1	≤ 0.1	0	0.05	0.06	ICNCM (1990)
Hanna Mining and Smelting Operations, Oregon, USA	0	<1.0	0	0	0	0	ICNCM (1990)
Alloy manufacturing and grinding							
Henry Wiggin Alloy Company, Hereford, UK	0	<1.0	0	<1	0.01	0.01	ICNCM (1990)
Huntington Alloys, Inc., West Virginia, US USA (after 1946)	0	0 – 0.45	0 – 0.05	0 – 0.26	0.03	0.03	ICNCM (1990)
Nickel Alloy Workers from 13 Plants, USA	0	0.01 – 1.9	0	<0.01 – 1.0	0.01	0.01	Sivulka and Seilkop (2009)
Barrier manufacturing							
Gaseous Diffusion Plant, Oak Ridge, Tennessee, USA	0	0	0	< <0.1 – 1.8	0.02	0.01	ICNCM (1990)
Nickel plating							
Nickel Plating Factory, Birmingham, Midlands, UK	0	0	<0.01 – 0.08	0	0.04	0.04	TERA (1999)

Table 3. continued on next page

Table 3. Continued.

Industry sector/cohort[a]	Estimated exposures (mg Ni/m³)				Soluble nickel equivalents		
	Sulfidic	Oxidic	Soluble	Metallic	Alveolar	Interstitial	Reference
	Exposures (mg Ni/m³)						
Animal Studies	Sulfidic	Oxidic	Soluble	Metallic			
LOAEC(HEC) [d]	0.36 – 2.32	3.47 – 22.1					
NOAEC(HEC) = MTD(HEC) [e]			0.38 – 0.95	1.82 – 8.47			

[a]See text for definitions of cohorts
[b]As calculated by ICNCM (1990).
[c]As calculated by Grimsrud et al. (2000).
[d]The lowest observable adverse effect concentration (LOAEC) is the lowest dose at which a statistically significant increase in tumor incidence was observed.
[e]The no observable adverse effect concentration (NOAEC) for rat tumors is the maximum tolerated dose (MTD) if no increase in tumor incidence was observed at any dose.

material for the enrichment of uranium, and was exposed solely to metallic nickel.

ICNCM (1990) found evidence that respiratory cancer risks could potentially be attributed to sulfidic and oxidic nickel together, oxidic nickel alone, and to water-soluble nickel. ICNCM (1990) reported no evidence of risks with metallic nickel. Updated analyses of several of these cohorts conducted since the ICNCM (1990) report have been published (e.g., Easton et al., 1992; Andersen et al., 1996; Grimsrud et al., 2002, 2003; Sorahan and Williams, 2005; Anttila et al., 1998). There have also been a number of analyses of workers in the nickel-producing and nickel-using industries since that time. These include studies of high nickel alloy workers in the USA (exposed to low levels of oxidic and metallic nickel), Canadian hydrometallurgical refinery workers (exposed to high levels of metallic nickel), and nickel platers in the UK (exposed solely to water-soluble nickel) (Arena et al., 1998, 1999; Egedahl et al., 2001, Pang et al., 1996).

Below we describe the key epidemiological studies that address the association between the forms of nickel that have been assessed in animal studies (i.e., sulfidic, oxidic, water-soluble, and metallic nickel) and lung cancer risk. This is followed by a comparison of workplace exposures in these studies to those in the animal studies described above and, based on this comparison, an analysis of whether human data support the nickel ion theory and/or the nickel ion bioavailability model.

9.1. Nickel refining operations with high insoluble and water-soluble nickel exposures

In several sulfidic nickel ore refining and processing operations, exposures to insoluble nickel (sulfidic and/or oxidic) were very high, and water-soluble nickel exposures were likely high as well (Table 3). Co-exposures to sulfidic, oxidic, and water-soluble nickel almost always occurred, and mainly in plants and processes that are no longer in existence (ICNCM, 1990). In addition, men working with one process may have been exposed to nickel from other processes in close proximity, particularly when processes took place in the same building (e.g., Harjavalta prior to 1973 as described by Antilla et al. [1998] and Kristiansand until at least 1978 as described by ICNCM [1990]).

The highest lung cancer risks in sulfidic ore refining and processing workers were reported among linear calcining, grinding, and milling workers at Clydach hired before 1930, with less than a year in hydrometallurgy, copper plant, or furnace work (standardized mortality ratio [SMR] = 725, $p < .001$; see Table 4). Clydach copper plant workers hired before 1930 (with less than 1 year in calcining or furnace operations before 1959 or work in hydrometallurgy departments), who were exposed to high levels of oxidic and water-soluble nickel but negligible amounts of sulfidic nickel, also demonstrated an increased lung cancer risk (SMR = 317, 95% confidence interval [CI]: 185–507). Risks were also elevated among men at obsolete operations in Canada, including the Copper Cliff, Ontario sinter plant (SMR = 307, 95% CI: 238–396) and the Port Colborne leaching, calcining, and sintering department (SMR = 239, 95% CI: 187–302).

ICNCM (1990) noted some evidence of increased lung cancer risk in Kristiansand roasting, smelting, and calcining workers (with less than one year of electrolysis exposure) who were exposed primarily to oxidic nickel (SMR = 225, 95% CI: 122–377), but the exposure-response relationship was weak. There was also some evidence that risk diminished with reductions in atmospheric oxidic nickel levels related to changes in refinery processes. A reanalysis of these data using a department-time-exposure matrix, which led to similar but slightly lower exposure estimates overall (Grimsrud et al., 2000), also found an increased lung cancer risk (standardized incidence ratio [SIR] = 330, 95% CI: 180–560). Strengths and limitations of this exposure matrix are discussed in Section 9.2.

In Clydach workers, lung cancer risks were associated with cumulative exposure to sulfidic nickel after adjustment for the levels of oxidic, soluble, and metallic nickel exposure. This was not the case at Kristiansand or among Huntington Alloys workers, some of whom were exposed to nickel from a calcining operation that was dismantled in 1946. ICNCM (1990) suggested this could be because of lower exposures to sulfidic nickel in Kristiansand (average <1.3 mg Ni/m³) and Huntington (4 mg Ni/m³ in calcining before 1947; no sulfidic nickel in other departments) than in Clydach (6–12 mg Ni/m³ in

linear calcining and nickel plant cleaning), or because of a combination of high sulfidic and water-soluble nickel levels at Clydach, but not at the other locations. Thus, although lung cancer risks were clearly elevated in workers in nickel refining operations with high insoluble and water-soluble nickel exposures, it is not evident to which forms of nickel the risks were attributable.

9.2. Nickel refining operations with low insoluble and high water-soluble nickel exposures

ICNCM (1990) concluded that water-soluble nickel (for which exposures always occurred in the presence of sulfidic or oxidic nickel) could increase respiratory cancer risk based primarily on the Kristiansand and Clydach cohorts. ICNCM (1990) estimated water-soluble nickel exposures to be 0.3–5 mg Ni/m^3 (sometimes >5 mg Ni/m^3) and sulfidic and oxidic nickel exposures to be generally <1.3 mg Ni/m^3 in Kristiansand electrolysis workers (with less than 1 year of roasting, smelting, or calcining exposure; Table 3). In contrast, Grimsrud et al. (2000) estimated that water-soluble nickel exposures ranged from 0.025 to 0.17 mg Ni/m^3 and that oxidic and sulfidic exposures were <0.016 mg Ni/m^3 in these same workers. ICNCM (1990) estimated exposure based primarily on the subjective judgments of retired personnel. Grimsrud et al. (2000) estimated exposure using a department-time-exposure matrix that was based on approximately 5900 personal measurements from 1973 to 1994 and over 500 stationary samples from prior to 1973, subjective judgments of experienced company staff, and various extrapolations. Although the Grimsrud et al. (2000) estimates are based on actual measurements, these measurements were primarily taken in the latter years of operation. A speciation method commonly used at that time has been recently reported to have the potential to overestimate water-soluble nickel and underestimate insoluble nickel (reviewed by Goodman et al., 2009). In addition, the supralinear exposure-response functions described by Grimsrud et al. (2002) based on these data suggest an underestimation of exposures at the low doses. Finally, as mentioned above, co-exposures to other forms of nickel would have come from other processes in close proximity to the electrolysis workers. Regardless, based on either the ICNCM (1990) or the Grimsrud et al. (2000) exposure estimate, lung cancer risks were strongly associated with working in electrolysis (with no roasting, smelting, or calcining exposure) in Kristiansand (SMR = 385, 95% CI: 259–549 based on ICNCM [1990] estimate; SIR = 510, 95% CI: 320–770 based on Grimsrud et al. [2000] estimate).

In the cross-classification analyses of lung cancer mortality data from Clydach, water-soluble nickel was also associated with increased risks of lung cancer in the presence of high levels of oxidic and sulfidic nickel ($p = .003$) and low levels of sulfidic nickel and high levels of oxidic nickel ($p = .024$). Unlike Kristiansand, however, this association did not hold if oxidic levels were low ($p > .9$). Based on the results of these analyses, ICNCM (1990) concluded that there was evidence that water-soluble nickel accentuated risks from other forms of nickel.

This hypothesis was also examined by ICNCM (1990) in its comparison of risks in Clydach copper plant workers exposed to high levels of oxidic nickel and 0.8–1.5 mg Ni/ m^3 water-soluble nickel (SMR = 317, 95% CI: 185–507) to those in the hydrometallurgy department with low levels of oxidic nickel but similar levels of water-soluble nickel (SMR = 196, 95% CI: 79–404). The differences in risks for workers in the two departments were not statistically significantly different, but risks for copper plant workers tended to be higher. ICNCM (1990) therefore concluded that risks in the copper plant could have been attributable to oxidic or water-soluble nickel or both (sulfidic nickel levels were negligible). Still, ICNCM (1990) suggested the lack of lung cancer risks without co-exposures to insoluble nickel in the cross-classification analysis could help explain the overall lack of risk observed in Port Colborne electrolysis workers with no sintering experience (SMR = 88, 95% CI: 53–137). Contrary to earlier estimates, it appears that both water-soluble and insoluble exposures were higher at Port Colborne than at Kristiansand (water-soluble nickel: <0.3 mg Ni/m^3 in general areas and 1–3 mg Ni/m^3 in "high exposure" areas associated with the pumping of anode slimes and washing of anode scrap; insoluble nickel: <0.5 mg Ni/m^3 in general areas and <0.8 mg Ni/m^3 in the high exposure areas).

Anttila et al. (1998) examined cancer risks in workers in a refinery and a smelter in the Outokumpu Oy nickel refinery in Harjavalta, Finland. These investigators found increased risks of lung cancer in both the refinery (SIR = 338, 95% CI: 124–736) and the smelter (SIR = 200, 95% CI: 107–342) when a latency of ≥20 years was assumed. Because nasal cancer risks were only elevated in the refinery, where primary exposures were to nickel sulfate, the authors suggested that cancer risks were likely attributable to water-soluble nickel. Although it is true that water-soluble nickel exposures were significant only in the refinery, interpretation of the role of water-soluble nickel in inducing the excess lung cancer risks is complicated by the fact that until 1973, grinding and leaching operations, which generated substantial levels of insoluble nickel, were in the same building where the electrolytic operations took place, likely resulting in co-exposure of electrolytic refinery workers to both water-soluble and insoluble nickel forms. Nearly all of the observed excess risk occurred in workers hired during this period of mixed water-soluble and insoluble nickel exposure, as all six lung cancer cases were hired before 1975. Furthermore, two of the lung cancer cases had also worked in the smelter, where they would have been exposed to insoluble nickel. Measurements of water-soluble nickel during the period 1979–1981 averaged 0.25 mg Ni/m^3, but measurements from the first 15 years of the refinery averaged 0.5 mg Ni/m^3 inhalable nickel. Anttila et al. (1998) suggested that water-soluble nickel was responsible for the risk observed in the refinery workers,

Table 4. Representative lung cancer risk estimates in nickel industry workers.

Industry sector/cohort[a]	Estimated exposures [b]		Soluble nickel equivalents[c]		Lung cancer		
	Sulfidic	Oxidic	Alveolar	Interstitial	Risk	Risk estimate	Reference
Refining operations with high insoluble and water-soluble nickel exposures							
Linear Calcining Department, Mond/INCO Refinery, Clydach, Wales					↑	SMR = 725, $p < 0.001$	ICNCM (1990)
Copper Plant, Mond/INCO Refinery, Clydach, Wales					↑	SMR = 317, 95% CI: 185–507	ICNCM (1990)
Sinter Plant, Copper Cliff, Ontario, Canada					↑	SMR = 307, 95% CI: 238–396	ICNCM (1990)
Leaching, Calcining, and Sintering, Port Colborne, Ontario, Canada					↑	SMR = 239, 95% CI: 187–302	ICNCM (1990)
Roasting, Smelting, and Calcining, Falconbridge Nickel Refinery, Kristiansand, Norway					↑	SMR = 225, 95% CI: 122–377	ICNCM (1990)
Roasting, Smelting, and Calcining, Falconbridge Nickel Refinery, Kristiansand, Norway					↑	SIR = 330, 95% CI: 180–560	Grimsrud et al. (2003)
Huntington Alloys, Inc., West Virginia, USA (before 1947)					–	SMR = 97, 95% CI: 76–122	ICNCM (1990)
Refining operations with low insoluble and high water-soluble nickel exposures							
Electrolysis, Falconbridge Nickel Refinery, Kristiansand, Norway					↑	SMR = 385, 95% CI: 259–549	ICNCM (1990)
Electrolysis, Falconbridge Nickel Refinery, Kristiansand, Norway					↑	SIR = 510, 95% CI: 320–770	Grimsrud et al. (2003)
Hydrometallurgy, Mond/INCO Refinery, Clydach, Wales					–	SMR = 196, 95% CI: 79–404	ICNCM (1990)
Electrolysis, Port Colborne, Ontario, Canada	?				–	SMR = 88, 95% CI: 53–137	ICNCM (1990)
Refinery, Outokumpu Oy, Harjavalta, Finland					↑	SIR = 338, 95% CI: 124–736[d]	Anttila et al. (1998)
Smelter, Outokumpu Oy, Harjavalta, Finland					↑	SIR = 200, 95% CI: 107–342[d]	Anttila et al. (1998)
Refining operations with high metallic nickel exposures							
Hydrometallurgy, Saskatchewan, Alberta, Canada					–	SMR = 67, 95% CI: 24–146	Egedahl et al. (2001)
Sulfidic ore mining and smelting with low insoluble and water-soluble nickel exposures							
Mining, Milling, Smelting Operations, Falconbridge, Ontario, Canada					↑	SMR = 135, 95% CI: 111–162 [e]	ICNCM (1990)
Mining, Milling, Smelting Operations, INCO, Ontario Canada	?				↑	SMR = 111, 95% CI: 102–121 [e]	ICNCM (1990)
Mining and smelting of lateritic ores							
Societe le Nickel Mining and Smelting Operations, New Caledonia					–	OR = 0.9, 95% CI: 0.5–1.8	Menvielle et al. (2003)
Hanna Mining and Smelting Operations, Oregon, USA[f]					–	SMR = 113, 95% CI: 45–233	ICNCM (1990)

Table 4. continued on next page

Table 4. Continued.

Industry sector/cohort[a]	Estimated exposures [b]		Soluble nickel equivalents[c]		Lung cancer		Reference
	Sulfidic	Oxidic	Alveolar	Interstitial	Risk	Risk estimate	
Alloy manufacturing and grinding							
Henry Wiggin Alloy Company, Hereford, UK					–	SMR = 87, 95% CI: 67–111	Sorahan (2004)
Huntington Alloys, Inc., West Virginia, USA (after 1946)[f]					–	SMR = 98, 95% CI: 59–153	ICNCM (1990)
Nickel Alloy Workers from 13 Plants, USA					–	RR = 1.01, 95% CI: 0.95–1.08 [g]	Arena et al. (1998)
					↑	RR = 1.13, 95% CI: 1.05–1.17 [h]	
Powder Metallurgy, Nickel Alloy Workers from 13 Plants, USA					–	RR = 0.77, 95% CI: 0.25–1.61	Sivulka (2005)
Barrier Manufacturing							
Gaseous Diffusion Plant, Oak Ridge, Tennessee, USA					–	SMR = 54, 95% CI: 25–103	ICNCM (1990)
Nickel plating							
Nickel Plating Factory, Birmingham, Midlands, UK					–	SMR = 108, 95% CI: 54–194	Pang et al. (1996)

[a]See text for definitions of cohorts. The same cohorts of roasting, smelting, and calcining workers, as well as electrolysis workers, at Falconbridge Nickel Refinery in Kristiansand, Norway were analyzed by both ICNCM (1990) and Grimsrud et al. (2003).
[b]Shaded cells indicate that exposures are greater than the human equivalent concentration (HEC) corresponding to the lowest observable adverse effect concentration (LOAEC) for tumors in rats.
[c]Soluble nickel equivalent values: <0.1 mg Ni/m^3—no shading; 0.1 to ≤1 mg Ni/m^3—light shading; >1 mg Ni/m^3—dark shading.
[d]Assuming a 20 year latency.
[e]Risks likely attributable to higher smoking rate in exposed population relative to reference population (Seilkop and Oller, 2003).
[f]For men working in "high" nickel-exposure jobs (0.1–1 mg Ni/m^3); speciation was assumed to be entirely of nickel oxide, although low amounts of iron-nickel oxides and soluble nickel may have been present.
[g]Risk estimate based on comparison with the local population.
[h]Risk estimate based on comparison with the US population.

but the role of co-exposure to insoluble nickel forms with concentrations in the range of 0.06–0.4 mg Ni/m^3 cannot be dismissed, particularly when one considers that smelter workers in the same plant with exposure to poorly soluble nickel compounds exhibited an increased lung cancer incidence.

In sum, associations between water-soluble nickel exposure and lung cancer risk were noted in several studies of sulfidic ore refining and processing workers. There is a strong possibility that risks attributed to water-soluble nickel could in fact be due to another form of nickel, or it could be that water-soluble nickel accentuates risks of other nickel forms, acting through a non-genotoxic mechanism. The epidemiological data alone are not robust to definitively assess this in humans.

9.3. Mining and smelting of sulfidic ores with low insoluble and water-soluble nickel exposures

Exposures to nickel were quite low (<1 mg Ni/m^3) in Falconbridge Ontario mining, milling, and smelting workers, as well as in INCO Ontario non-sinter workers (ICNCM, 1990). At Falconbridge, workers were exposed to nickel in the form of minerals (pentlandite and pyrrhotite) in mining and milling, and to sulfidic nickel, oxidic nickel, nickel sulfate, and nickel-copper alloy in the smelter (ICNCM, 1990). Lung cancer risks were elevated overall (SMR = 135, $p = .001$), and primarily in miners (SMR = 144, $p < .01$), surface workers (SMR = 192, $p < .01$), and smelter workers (SMR = 143, $p < .05$) with 15 or more years since first exposure. Because risks were similar among other hard-rock miners with no nickel exposures, ICNCM (1990) concluded that the attribution of increased risk to nickel at Falconbridge was questionable.

INCO Sudbury non-sinter workers may have been exposed to slightly higher levels of each form of nickel than Falconbridge non-sinter workers. INCO workers who did not work at the Copper Cliff or Coniston sinter plants with 15 or more years since first exposure had a modest increase in lung cancer risk (SMR = 111, 95% CI: 102–121). Most of the risk occurred among copper refinery and mining workers.

Seilkop and Oller (2003) analyzed smoking rates among blue collar workers in Canada and suggested the increased lung cancer risks in both of these cohorts were likely attributable to a higher smoking rate in the workers versus the reference population.

9.4. Nickel refining operations with high metallic nickel exposures

In contrast to soluble nickel, for which there are no major cohorts lacking other types of nickel exposure, there are two major groups of workers (totaling ~1500 individuals) for whom exposures to metallic nickel predominate: uranium barrier workers (discussed in Section 9.7) and hydrometallurgical refinery workers. Seven hundred eighteen hydrometallurgical refinery workers with 18,237 person-years of follow-up were exposed predominantly to nickel concentrates and high levels of metallic nickel (mean 2–4 mg Ni/m^3) in Fort Saskatchewan, Alberta, Canada (Egedahl et al., 2001). No excess risks of lung and bronchus cancers were found among these workers (SMR = 67, 95% CI: 24–146). After 24,905 person-years of follow-up through 2003, Egedahl and Collins (2009) reported similar results (SMR = 74, 95% CI: 38–129).

9.5. Mining and smelting of lateritic ores

Whereas respiratory cancer risks were increased in sulfidic ore smelting and refining workers, this was not the case for lateritic ore workers, for which there are low exposures to oxidic nickel and de minimus exposures to sulfidic nickel (ICNCM, 1990; Goldberg et al., 1994; Menvielle et al., 2003). In Societé le Nickel mining and smelting operation workers followed from 1978 to 1984, ICNCM (1990) found no evidence that nickel exposure was associated with lung cancer (relative risk [RR] = 0.90, $p > .05$) or upper respiratory tract cancer (RR = 1.40, $p > .05$). The results for lung cancer were confirmed by Goldberg et al. (1994), who followed these workers to 1987, for 87,957 person-years of follow-up (exposure <20 years [$n = 28$], RR = 0.7, 95% CI: 0.5–0.9; exposed 11–20 years [$n = 16$], RR = 0.4, 95% CI: 0.2–0.6; exposed >20 years [$n = 10$], RR = 0.4, 95% CI: 0.2–0.8). In a case-control study of 228 lung cancer cases diagnosed between 1993 and 1995 and 305 population controls in New Caledonia, Menvielle et al. (2003) also found no evidence of statistically significant increased lung cancer risks associated with nickel (odds ratio [OR] = 1.3, 95% CI: 0.7–2.6) or iron and nickel oxides (OR = 0.9, 95% CI: 0.5–1.8).

In the Hanna mining and smelting operations in Oregon, USA, workers were primarily exposed to <1 mg Ni/m^3 of oxidic nickel. Lung cancer risk was increased overall (SMR = 147, 95% CI: 97–213), but was attributable to men with less than 1 year of exposure to nickel (SMR = 265, $p < .05$). Men with 15 or more years of exposure did not have an increased risk (SMR = 124, $p > .05$), nor did men with "high exposure" jobs (exposures of 0.1–1 mg Ni/m^3, SMR = 113, 95% CI: 45–233). Thus, it is unlikely that the overall association was causal.

ICNCM (1990) suggested the difference in risk between sulfidic ore and lateritic ore processing operations could be due to (1) the presence of nickel-copper oxide in sulfidic ore and the lack of copper in lateritic ore (lateritic ores contain complex iron oxide/silicate compounds); or (2) higher exposures to oxidic nickel during sulfidic ore refining (10–100 mg Ni/m^3 in Clydach and >8 mg Ni/m^3 in Kristiansand) compared to during lateritic ore mining and smelting (<1 mg Ni/m^3 in both the lateritic ore operations). The latter is consistent with the NTP (1996c) study, in which human equivalent concentrations of 3.5–22 mg oxidic Ni/m^3 are the lowest at which tumors occurred (see Table 6).

9.6. Alloy manufacturing and grinding

Whereas exposure to oxidic nickel always occurred with co-exposure to sulfidic nickel in the sulfidic ore nickel-producing industry, this was not the case for

the nickel-using industry. There are many nickel-using industries, but epidemiological studies conducted to date are primarily of individuals working in the manufacture of nickel alloys, stainless steel (SS), plates, and uranium barrier material. SS and nickel-containing alloy manufacture accounts for 90% of the nickel in the using industries. Exposures are primarily to oxidic and very low levels of metallic nickel, and this is also the case for nickel grinding (Sivulka, 2005). All of these nickel-using industries also involve exposures to other substances, including other metals and asbestos, which may be carcinogenic. In addition, the form of oxidic nickel in these industries is generally copper-free, in contrast to that used in the sulfidic ore nickel-producing industry, which is a nickel-copper oxide.

Table 5. Workplace particle size distribution (PSD) for various nickel substances.

Work place	PSD[a] (μm) Sample	MMAD	GSD	γ	Main nickel substances for which workplace PSD is of relevance
Electrolysis	1	34.18	3.39	0.95	Nickel sulfate
	1	1.42	2.89	0.05	Nickel chloride
	2	50.68	1.10	0.68	Metallic nickel
	2	10.21	2.27	0.32	
Matte grinding	1	19.00	2.53	0.94	Nickel oxide
	1	3.79	1.10	0.06	Nickel subsulfide
	2	50.67	1.10	0.68	Metallic nickel
	2	11.46	2.38	0.32	
Roasting/ smelting	1	46.40	2.75	0.96	Nickel oxide
	1	42.45	17.4 [b]	0.04	Nickel subsulfide
	2	61.20	3.52	1.00	Metallic nickel
	2	50.90	3.01	0.00	

[a]Yu et al., 2001. These distributions are relevant for producers and users as described by Oller and Oberdörster (2010).
[b]The Multiple Path Particle Deposition (MPPD) program assumed a maximum geometric standard deviation (GSD) of 5, instead of the actual 17.4, for the calculation of the human equivalent concentration (HEC). This PSD measurement was not used in the derivation of HECs in Table 6 because it would have led to an overestimation of exposure.

Lung cancer risks were assessed in workers in a nickel alloy manufacturing plant in Hereford, UK, by Cox et al. (1981), ICNCM (1990), and Sorahan (2004). None of the studies reported increased risks of respiratory cancers. Exposures to oxidic, metallic, and water-soluble nickel were <1, <0.2, and <0.05 mg Ni/m^3, respectively. The most recent study included 1999 male workers first employed between 1953 and 1992, who worked for the company for at least 5 years. An increased risk of lung cancer was not observed (SMR = 87, 95% CI: 67–111; Sorahan, 2004).

In 1947, calciners in a nickel alloy plant in Huntington, West Virginia, were dismantled and the site became strictly an alloy producer. The cohort of 1353 workers who were hired after 1946 were exposed to copper-free oxidic nickel (0–0.45 mg Ni/m^3), metallic nickel (0–0.26 mg/m^3), and minute amounts of water-soluble nickel (0–0.05 mg Ni/m^3). ICNCM (1990) reported no increased risk of lung cancer (SMR = 98, 95% CI: 59–153).

In the largest alloy worker cohort, Arena et al. (1998) studied approximately 31,000 workers in 13 plants (including the Huntington, West Virginia, plant) who were involved in the production of high nickel alloys for at least 1 year between 1956 and 1967. During the 1940s to 1960s (the period of highest employment and most relevance to lung cancer induction relative to study follow-up), nickel exposures for various work areas ranged from 0.13 to 2.22 mg Ni/m^3 (average 0.73 mg Ni/m^3; Sivulka and Seilkop, 2009). The entire cohort contributed 800,373 person-years at risk. Seventy-nine percent of the workers were followed for ≥25 years since their first

Table 6. Human equivalent concentrations (HECs) of rat nickel exposures for the thoracic region of the respiratory tract.

Rat experimental exposure[a]							HEC$_w$ (mg Ni/m^3)[c]	
Substance	NOAEC or LOAEC [d]	Concentration (mg Ni/m^3)	PSD (μm) MMAD	GSD	Work area where particular forms of nickel predominate	Rat and human thoracic deposition/ unit surface area (ng/ cm^2)[b]	Based on equivalent deposited doses/ day[e]	Based on equivalent retained doses/long-term[f]
Nickel sulfate	NOAEC	0.11	2.25	2.08	Electrolysis	0.099	0.38 – 0.51	0.71 – 0.95
Metallic nickel	NOAEC	0.4	1.70	2.16	Electrolysis Matte grinding Roasting/smelting	0.44	1.82 – 5.64	2.73 – 8.47
Nickel subsulfide	LOAEC	0.1	2.17	2.34	Matte grinding Roasting/smelting	0.112	0.36 – 1.24	0.68 – 2.32
Nickel oxide	NOAEC	0.5	2.21	1.97	Matte grinding	0.54	1.77 – 5.99	3.31 – 11.24
	LOAEC	1.0	2.23	1.89	Roasting/smelting	1.07	3.47 – 11.78	6.51 – 22.08

[a]NTP (1996a, 1996b, 1996c); Oller et al. (2008).
[b]Deposition/unit surface area in humans that corresponds to the same deposition/unit surface area in rats.
[c]Approaches applied to derive human equivalent concentrations for the workplace (HEC$_w$) are described by Oller and Oberdörster (2010).
[d]The no observable adverse effect level (NOAEC) for rat tumors is the maximum tolerated dose (MTD) if no increase in tumor incidence was observed at any dose. The lowest observable adverse effect level (LOAEC) is the lowest dose at which a statistically significant increase in tumor incidence was observed.
[e]The comparison was made at daily deposited dose/surface area level; no assumptions were made for modifying effects of differences in retention half-life between rats and humans or duration of exposure.
[f]The comparison was made at long-term retained dose/surface area level; the same retention half-life for rats and humans was assumed; a correction for duration of exposure was applied (lifetime for rats; less than lifetime for humans).

occupational exposure in the high nickel alloys industry, and 24,202 person-years were distributed among workers with 20 or more years of cumulative employment. Arena et al. (1998) reported no excess lung cancer risk when the entire cohort was compared to the local population (RR = 1.01, 95% CI: 0.95–1.08), but a small increased risk compared to the US population (RR = 1.13, 95% CI: 1.05–1.17). No exposure-response association with duration of exposure was seen independent of the comparison used. In the powder metallurgy department, where metallic nickel exposures were 3 mg Ni/m^3, there was no evidence of excess lung cancer risks among 216 workers (RR = 0.77, 95% CI: 0.25–1.61; Sivulka, 2005). Lung cancer risks were also not elevated in a subcohort of women from this population (Arena et al., 1999).

Moulin et al. (1990, 2000) studied two cohorts of stainless and alloyed steel workers in France. Moulin et al. (1990) followed 2269 SS and ferrochromium workers from 1952 to 1982. Lung cancer risks for the whole cohort were not elevated (SMR = 1.40, 95% CI: 0.72–2.45) but an excess was found among "exposed" workers (SMR = 2.04, 95% CI: 1.02–3.64). Based on a nested case-control study, the authors attributed the excess risk to former polycyclic aromatic hydrocarbon (PAH) exposures in the ferrochromium production workshops rather than to exposures in the SS manufacturing areas. The association between lung cancer and exposure to iron oxides, chromium, and/or nickel compounds was assessed by Moulin et al. (2000) in 4288 male and 609 female French workers involved in the production of stainless and alloyed steel from 1968 to 1992. Lung cancer risks were not significantly elevated (SMR = 1.19, CI: 0.88–1.55). In a nested case-control study of 54 cases and 162 controls, lung cancer risks were not associated with chromium and/or nickel exposure (OR = 1.18, CI: 0.62–2.25).

Nickel grinding workers are exposed to similar nickel forms as alloy workers are, although levels of exposure are likely to be much lower. Svensson et al. (1989) reported that exposures to nickel from 1974 to 1980 were 0.05 mg Ni/m^3 during grinding and 0.005 mg Ni/m^3 during polishing. Jakobsson et al. (1997) evaluated the cancer risk of 727 Swedish workers between 1952 and 1992 during the grinding of SS for the production of SS sinks and saucepans. They reported no increased incidence of lung cancer (SIR = 60, 95% CI: 20–120). Hansen et al. (1996) investigated cancer incidence in 10,059 Danish metal workers employed between 1964 and 1984. Of these workers, 521 were SS grinders employed between 1964 and 1985. These workers did not have statistically significant elevated respiratory cancer risks (SIR = 157, 95% CI: 78–281). Svensson et al. (1989) studied 1164 male workers who were exposed to the dust of grinding materials, grinding agents, and SS in an industry that produced objects from SS between 1927 and 1981. These investigators observed no cases of respiratory cancer (4.7 expected). Together, these studies do not provide evidence for an increased risk of respiratory cancer in nickel grinding workers.

9.7 Uranium barrier manufacturing

Apart from powder metallurgy in nickel alloy production (see Section 9.5), uranium barrier manufacturing is the only nickel-using industry in which workers were exposed to metallic nickel alone (<1 mg Ni/m^3) and for which there are data regarding respiratory cancer risk. Cragle et al. (1984) and ICNCM (1990) assessed cancer risks in 813 barrier workers at the Oak Ridge Gaseous Diffusion Plant who worked between 1948 and 1953 and were followed through 1977, and found lung cancer risks were not elevated (SMR = 54, 95% CI: 25–103).

9.8 Nickel plating

Very few nickel workers were exposed solely to water-soluble nickel, with the exception of nickel platers. Pang et al. (1996) analyzed mortality in 284 UK male nickel platers who were first employed between 1945 and 1975, followed through 1993, and worked at least 3 months in the nickel-plating department. They were likely exposed to 0.08 mg Ni/m^3. The cohort was small and the mean duration of exposure was short (2.1 years), but follow-up was long (up to 48 years). Pang et al. (1996) found no evidence of increased lung cancer (11 observed cases, SMR = 108, 95% CI: 54–194).

There have been several studies of nickel/chromium platers, but co-exposures to chromium generally make it challenging to determine whether risks are attributable to chromium or nickel. In a mortality study of 2689 nickel/chromium platers in England and Wales between 1946 and 1983, increased risks of lung and bronchial cancers and nasal and laryngeal cancers were reported (Sorahan et al., 1987). In this study, 144 of 564 workers with some period of chrome bath employment had either separate or simultaneous periods of nickel bath employment. Based on regression and life table analyses, Sorahan et al. (1987) concluded that nickel was not a confounder of the association between the duration of chrome employment and risk of any of these cancers.

9.9 Analyses of specific forms of nickel

Several investigators have attempted to determine which specific forms of nickel were responsible for lung cancer risk, particularly in the Clydach and Kristiansand cohorts. ICNCM (1990) conducted several cross-classification analyses of Clydach, and Easton et al. (1992) modeled lung cancer risks at Clydach associated with cumulative exposure to oxidic, sulfidic, metallic, and water-soluble nickel based on the exposure estimates prepared and used by the ICNCM (1990). ICNCM (1990, p. 43) concluded that:

The results of the cross-classification analysis suggest that increased lung cancer risk was associated with exposure to sulfidic and, possibly, oxidic nickel. There was also a strong indication that soluble nickel exposure accentuated the risks associated with exposure to these substances, with little evidence that soluble nickel exposure alone resulted in increased lung cancer risk. There was no evidence to suggest that metallic nickel exposure resulted in increased lung cancer risk.

Similarly, Easton et al. (1992) suggested that models with and without water-soluble and metallic nickel explained lung cancer risks in Clydach workers equally well. When Easton et al. (1992) tested their models against observed risks in more recent employees, there was substantial lack of fit, leading the authors to conclude that they overestimated risks from metallic and water-soluble nickel and underestimated risks from sulfidic and oxidic nickel.

Grimsrud et al. (2002) analyzed lung cancer risks in a nested case-control study of the Kristiansand cohort and reported a statistically significant trend of increased risk with increased exposure to water-soluble nickel, but not other forms of nickel. Despite this, qualitatively similar trends were observed for all forms of nickel. As Grimsrud et al. (2002) suggested, nickel exposures were all "moderately to highly correlated." In addition, exposures to individual nickel species were based on measurements obtained with the Zatka method (Zatka et al., 1992) and then applied to each worker based on his job(s) at Kristiansand. Although the Zatka method was "the most extensively used method to estimate the proportion of various kinds of nickel species in workplace aerosol samples" (Sivulka et al., 2007), it has been shown to overestimate water-soluble nickel exposure while underestimating sulfidic nickel exposure (reviewed in Goodman et al., 2009; Oller et al., 2009). Also, because workers often held several jobs and worked in areas that were not always physically separated, each worker was likely exposed to nickel forms not associated with his specific job(s), including electrolysis workers, who were assumed to be exposed to primarily water-soluble nickel, but may have been exposed to insoluble nickel forms as well (Haber et al., 2000).

9.10. Summary of epidemiology and animal carcinogenicity data

To test whether the nickel ion theory or the nickel bioavailability model best describes the epidemiology and animal carcinogenicity data, we compared exposures and risks in humans to those in animals. Table 3 shows occupational exposure estimates that are based on data reported by ICNCM (1990), Grimsrud et al. (2000), Anttila et al. (1998), Egedahl (2001), Sivulka and Seilkop (2009), and TERA (1999). Although there are many uncertainties associated with these estimates, many of which are described above, the relative exposure of each group of workers to each nickel form is likely to be reasonable.

For comparison, the exposure levels from the animal studies are also shown in Table 3. Because the particle size distributions (PSDs) of aerosols in the animal studies differed from those in the workplace, we converted the animal LOAECs for tumor induction (for sulfidic and oxidic nickel) and NOAECs (for water-soluble and metallic nickel) to human equivalent concentrations (HECs) using the PSDs for refinery workplaces described by Yu et al. (2001) and an approach based on the multiple-path particle deposition (MPPD) model for respiratory tract deposition in rats and humans as described by Oller and Oberdorster (2010; see Tables 5 and 6). The range of HECs calculated for each nickel form is shown in Table 3. The range of HECs reflect (1) calculations that were made based on equivalent daily deposited doses and those that were based on equivalent retained doses (which are appropriate if retention half-times in rats and humans are known or are expected to be similar); and (2) calculations made based on different particle size distributions measured at the worksites of relevance for each form of nickel (Table 6).

Table 3 also shows the exposure levels to soluble nickel equivalents calculated by taking the solubility of each form of nickel in synthetic lung fluids into account. Soluble nickel equivalents were estimated using the following equations, based on the ratio of the nickel ion release in alveolar or interstitial fluid of each nickel species to water-soluble nickel at 37°C after 24 hours, as described by Oller et al. (2009):

Soluble nickel equivalents based on alveolar fluid nickel ion release = Soluble + (6.7/137) × Sulfidic + (0.5/137) × Oxidic + (2.3/137) × Metal

Soluble nickel equivalents based on interstitial fluid nickel ion release = Soluble + (26/120) × Sulfidic + (0.7/120) × Oxidic + (1.6/120) × Metal

The soluble nickel equivalents based on alveolar fluid and interstitial fluid nickel ion release for each occupational cohort (based on the average values for each nickel form) are shown in Table 3. In Table 4, cells with soluble nickel equivalents <0.1 mg Ni/m^3 (low) are not shaded; those between 0.1 and 1 mg Ni/m^3 are shaded in light gray (medium); and those ≥1 mg Ni/m^3 are shaded in dark gray (high). Soluble nickel equivalents based on alveolar and interstitial fluid nickel ion release are generally similar and, with two exceptions, fall into the same category (low, medium, high) for each group of workers.

9.11. Nickel ion bioavailability model

The nickel ion bioavailability model predicts an increased incidence of lung cancer in workers exposed to high levels of sulfidic and certain oxidic nickel compounds, with no increased risks in workers exposed to water-soluble nickel compounds or metallic nickel alone. To assess whether the risks predicted by the nickel ion bioavailability model were realized in humans, we compared occupational exposures to sulfidic and oxidic nickel in all of the epidemiology studies reviewed here to the LOAECs for these substances in the animal studies described above (Table 3).

Table 4 shows representative lung cancer risks for each of the occupational cohorts, and each exposure to sulfidic or oxidic nickel higher than the respective minimum LOAEC(HEC) is shaded. In refining operations with high insoluble and water-soluble nickel exposures, lung cancer risks were consistently elevated, and sulfidic and oxidic nickel levels were higher than their respective rat LOAECs(HECs), except at Huntington Alloys before 1947, at which lung cancer risks were not

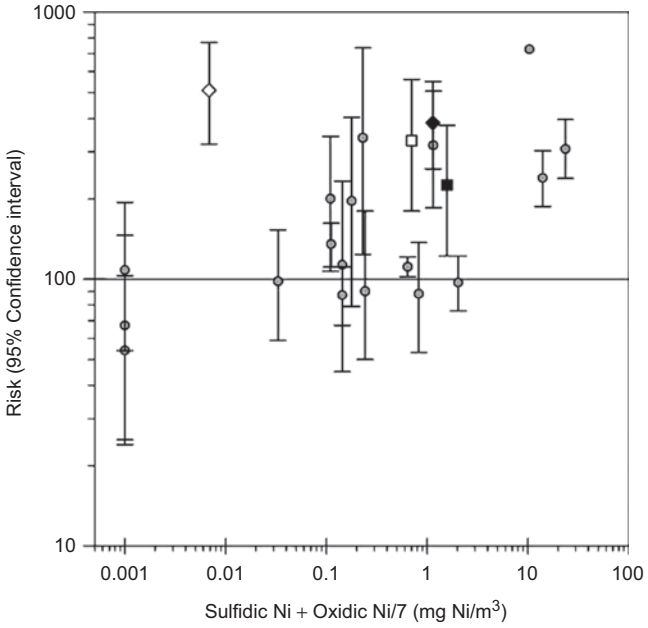

Figure 2. Insoluble nickel exposure and lung cancer risk.

elevated and oxidic nickel exposures were not higher than the LOAEC(HEC). In refining operations with low insoluble but high water-soluble nickel exposures, oxidic nickel exposures were always less than the LOAEC(HEC), although it should be noted that if the oxidic nickel forms present in the sulfidic ore refineries were Ni-Cu oxides with higher potency than green nickel oxides, increased cancer risks could have manifested even if exposures to oxidic nickel were below the LOAEC(HEC) for green NiO. Sulfidic nickel exposures were low, but higher than the rat LOAEC(HEC) in Harjavalta refinery workers and Kristiansand electrolysis workers (based on ICNCM [1990] exposure estimates, but not Grimsrud et al. [2000] exposure estimates). Sulfidic nickel exposures of Port Colborne electrolysis workers were reported as <0.8 mg Ni/m^3, so it is not possible to determine whether they were higher than the LOAEC(HEC). Sulfidic nickel exposures were not higher than the rat LOAEC(HEC) in Harjavalta smelter workers or Clydach hydrometallurgy workers. Among all of these workers in refining operations with low insoluble but high water-soluble nickel exposures, lung cancer risks were elevated in Kristiansand electrolysis workers and Harjavalta smelter and refinery workers. As discussed above, however, these workers may have been exposed to nickel from other processes in close proximity (Antilla et al., 1998; ICNCM, 1990).

Among sulfidic ore mining and smelting workers with both low insoluble and water-soluble nickel exposures (Falconbridge and INCO Ontario mining, milling, and smelting workers), exposure to oxidic nickel was always below the rat LOAEC(HEC) and sulfidic nickel exposure was below the rat LOAEC(HEC) at Falconbridge and below the limit of detection at INCO, although it was likely also below the rat LOAEC(HEC). Lung cancer risks were elevated in both cohorts, but smoking appears to be a likely contributor to these risks (Seilkop and Oller, 2003).

There were limited exposures to sulfidic and oxidic nickel at the Saskatchewan refinery, and lung cancer risks were not elevated. Workplace exposures to sulfidic and oxidic nickel in lateritic ore smelting, alloy manufacture and grinding, barrier manufacturing, and nickel-plating operations were all below the respective LOAECs(HECs). Risks for lung cancer were not elevated in any of these workplaces except among US alloy workers when compared to the US population, but not when compared to the local population.

As part of an Expert workshop convened by Toxicology Excellence for Risk Assessment (TERA; http://www.tera.org/Peer/NiBioavailability/), Steven Seilkop examined whether increases in exposure to sulfidic and/or oxidic nickel (which have the highest bioavailability) were associated with increased lung cancer risk. Specifically, he plotted lung cancer risk relative to the sum of the average estimated sulfidic nickel concentrations plus oxidic nickel concentrations divided by 7; the oxidic nickel exposures were divided by 7 to reflect the differential in tumorigenic potency of nickel oxide relative to nickel subsulfide observed in the NTP studies. Although there is considerable uncertainty regarding exposure measurements, as shown in Table 3 and Figure 2, it appears plausible that lung cancer risk increased with increased exposure to sulfidic and oxidic nickel. This is consistent with the nickel ion bioavailability model.

In summary, all workers with exposures to oxidic nickel higher than the rat LOAEC(HEC) were also exposed to sulfidic nickel at levels greater than the rat LOAEC(HEC), and had an increased lung cancer risk. Almost all workers with exposure to sulfidic nickel higher than the rat LOAEC(HEC) had an increased lung cancer risk; the exceptions are Huntington Alloy refinery workers before 1947 and possibly Port Colborne electrolysis workers (although their exposures are not clearly known), who did not have increased lung cancer risks. The majority of workers whose exposures to sulfidic and oxidic nickel were less than the rat LOAEC(HEC) did not have increased lung cancer risks. The exceptions are electrolysis workers at Kristiansand when risks were based on Grimsrud et al. (2000), but not ICNCM (1990) exposure estimates; Harjavalta smelter workers, who were likely co-exposed to other nickel forms; mining, milling, and smelting workers at Falconbridge and INCO, whose risks could have been attributable to smoking; and nickel alloy workers from the USA when risks were based on comparisons to the US population, but not when compared to the local population. The sensitivity analysis indicated that an exposure-response gradient was plausible. Taken together, these data suggest that lung cancer risks are increased in the presence of sulfidic and possibly oxidic nickel, and are not elevated in the absence of high levels of either of these nickel forms. These data are consistent with the nickel ion bioavailability model, which predicts an increased incidence of lung cancer in workers

exposed to high levels of sulfidic and certain oxidic nickel compounds.

9.12. Nickel ion theory

The nickel ion theory predicts an increased incidence of lung cancer in workers exposed to sufficiently high levels of any form of nickel, with risks proportional to the solubility of nickel. To assess whether risks calculated in the epidemiological studies are consistent with the nickel ion theory, we assessed whether risks were correlated with soluble nickel equivalents as described in Section 9.10.

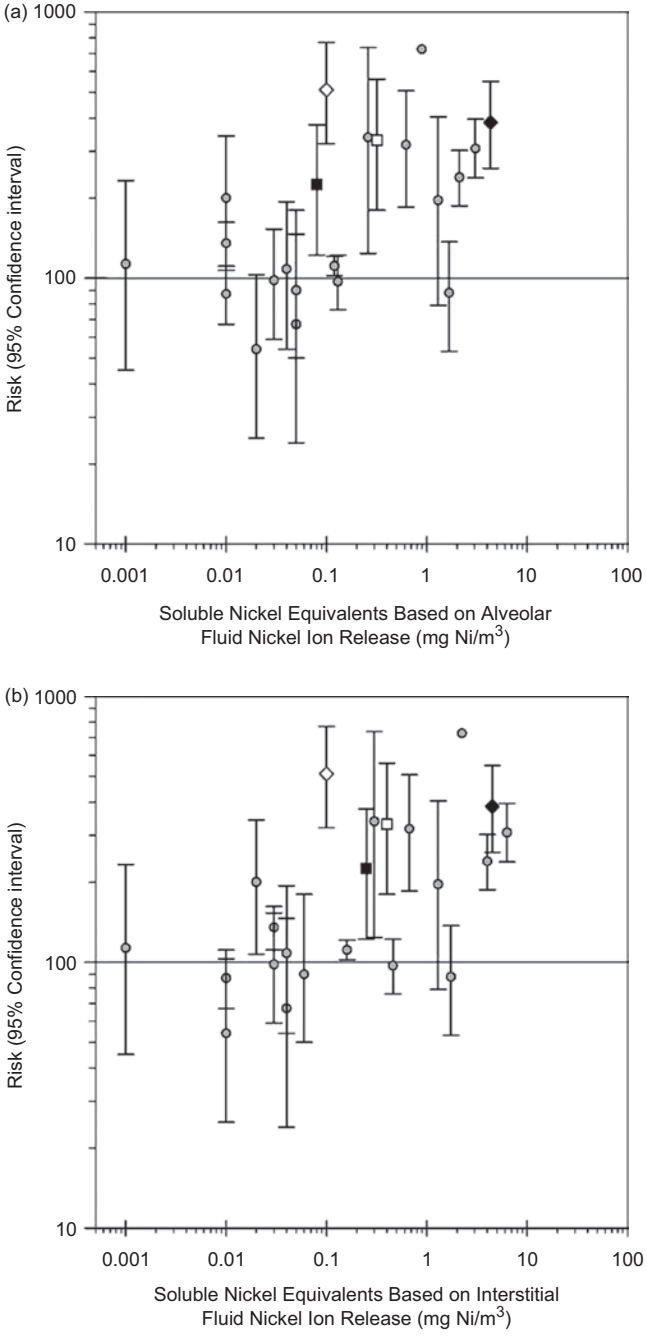

Figure 3. Soluble nickel equivalents based on (a) alveolar fluid or (b) interstitial fluid nickel ion release and lung cancer risk.

Estimates of soluble nickel equivalents were medium or high in all sulfidic ore refining operation workers except for Harjavalta smelter workers. They also might have been medium for INCO Ontario mining, milling, and smelting workers, but it is not likely because all nickel exposures were below the limit of detection. All other workers were exposed to low levels of soluble nickel equivalents.

Lung cancer risks were elevated in all workers exposed to medium or high levels of soluble nickel equivalents except for Huntington Alloy refinery workers (before 1947), Clydach hydrometallurgy workers, and Port Colborne electrolysis workers. There was no trend of higher risks with high versus medium exposures to soluble nickel equivalents. In all cases of increased lung cancer risks, workers were also exposed to levels of sulfidic nickel, and often oxidic nickel, higher than the rat LOAEC(HEC). In Clydach (hydrometallurgy) and probably Port Colborne workers, where risks were not elevated, exposures to sulfidic and oxidic nickel were below the rat LOAEC(HEC).

All workers exposed to low levels of soluble nickel equivalents were also exposed to levels of sulfidic and oxidic nickel below the respective LOAEC(HEC). Among these workers, lung cancer risks were only elevated in Harjavalta smelter workers; Falconbridge Ontario milling, mining, and smelting workers (although risks here and at INCO Ontario milling, mining, and smelting are likely due to smoking); and among US alloy workers when compared to the US population, but not when compared to the local population.

Seilkop, as part of the Expert workshop convened by TERA (http://www.tera.org/Peer/NiBioavailability/), examined lung cancer risk relative to the soluble nickel equivalents calculated based on nickel ion release in alveolar fluid or interstitial fluid (Table 3 and Figure 3). Once again, although there is considerably uncertainty in the exposure measurements on which the soluble nickel equivalents were based, the graphs suggest that it is plausible that lung cancer risk increases with increased exposure to soluble nickel equivalents. This is consistent with the nickel ion theory and with possible promoting effects of soluble nickel.

Overall, lung cancer risks are generally higher in workers exposed to medium or high levels of soluble nickel equivalents, but only in the presence of sulfidic, and often oxidic, nickel. All workers exposed to low levels of soluble nickel equivalents were also exposed to levels of sulfidic and oxidic nickel below their respective LOAEC(HEC). Except for the lack of risks in Clydach hydrometallurgy and Port Colborne electrolysis workers, these findings are generally consistent with the nickel ion theory.

9.13. Conclusions

The nickel ion theory purports that all forms of nickel should lead to increased lung cancer risks, with water-soluble nickel being the most carcinogenic, followed by sulfidic and metallic nickel, and then oxidic nickel. In contrast, the nickel ion bioavailability model predicts an

increased incidence of lung cancer in workers exposed to sulfidic and oxidic nickel compounds, but not in workers exposed to water-soluble nickel compounds alone or metallic nickel. Epidemiological data clearly support an association between nickel and lung cancer risk, and particularly some combination of sulfidic, oxidic, and/or water-soluble nickel or one of these forms of nickel alone. Robust cohorts with exposure solely to water-soluble nickel are lacking, but based on Clydach hydrometallurgy and Port Colborne electrolysis workers, it appears that the data are weakest regarding water-soluble nickel. Also, the available data on metallic nickel do not support a causal role, although it is difficult to draw conclusions based solely on epidemiological data. We cannot tease out which particular nickel form or forms are associated with risk. The epidemiological data tend to favor the nickel ion bioavailability model but are consistent with both the nickel ion theory and the nickel ion bioavailability model and cannot be used, in isolation, to determine which model is more appropriate.

10. Research needs

Although the currently available animal and mechanistic data support the nickel ion bioavailability model, there are several areas in which additional data would greatly improve our understanding of the processes that affect the bioavailability of the nickel ion from nickel-containing substances in respiratory epithelial cells.

Nickel-copper oxide exposures have been associated with lung cancer risk in refinery workers, but there have been no robust animal inhalation bioassays conducted with nickel-copper oxides. Data from such a study would corroborate the epidemiological data if increased incidences of lung tumors were observed with nickel-copper oxides, supporting a different carcinogenic potential for nickel-copper oxides compared to copper-free nickel oxides. A short-term inhalation study in which relative toxicity and estimated nickel burdens and clearance rates could be compared to those of nickel monoxide at the same exposure levels and duration could be useful as well. There are also limited data on the uptake and intracellular dissolution of copper-nickel oxides; such information would provide insight into the bioavailability of nickel-copper oxides that may support the findings from animal and human studies.

Much of the knowledge base for metallic nickel clearance is from animal studies that examined ultrafine particles (less than 0.1 μm in diameter) rather than the respirable-size particles used in the chronic carcinogenicity study with nickel metal powder. Further analyses of the lung burden data collected in the chronic study with metallic nickel would confirm the limited data on the clearance behavior of respirable-size metallic nickel particles from the 90-day studies.

The cellular uptake of nickel-containing substances in the respiratory tract are key areas in which more research is needed to better understand the bioavailability of nickel in the respiratory tract. The available data suggest that water-soluble nickel compounds are taken up by cells through ion-transport channels following extracellular dissolution, whereas particles of insoluble nickel-containing substances are taken up via endocytosis. Most of the studies on nickel uptake have been conducted with hamster cell lines (e.g., CHO cells or SHE cells), fibroblasts, or macrophages as surrogates for respiratory epithelial cells. Relative uptake studies of different nickel-containing substances in rat and human lung epithelial cells are necessary to provide quantitative data on the cellular uptake and disposition of particulate vs. dissolved nickel in the appropriate cell type. Such studies could determine if there is uptake of nickel into specific organelles or if there are other methods of nickel compartmentalization within lung epithelial cells. In addition, cell transformation studies that examine the effects of both uptake and intracellular dissolution can provide evidence of the relative ability of various nickel substances to become bioavailable at nuclear sites. The use of rat and human lung epithelial cells would also be preferred for these studies.

Several physiologically based pharmacokinetic (PBPK) and intracellular dosimetry models have been developed to describe lung deposition, clearance, and systemic distribution of nickel after inhalation exposures (Edelman and Roggli, 1989; Hsieh et al., 1999; Menzel et al., 1987; Oberdörster, 1989; Hack et al., 2007). Additional data related to the areas described above would be useful for the development of more robust models that consider differences in intracellular kinetics among nickel-containing substances after inhalation. For example, data from quantitative studies of nickel uptake into rat and human lung epithelial cells could be used in the intracellular dosimetry model developed by Hack et al. (2007) to compare the cellular dosimetry of nickel-containing substances from animal and human studies. The conditions under which the amount of nickel ions in the nucleus is the same for both nickel subsulfide and nickel sulfate hexahydrate could be identified in vitro and alterations in cell signaling proteins or gene expression could be compared. Such comparisons between rat and mouse lung epithelial cells could also help explain the lack of lung tumors in the mouse bioassays. Nickel sulfate hexahydrate could also be compared to cobalt sulfate heptahydrate in this manner to gain insight into the reason water-soluble nickel is not carcinogenic in rats after inhalation but water-soluble cobalt is. After in vivo validation, these data could inform risk assessments by allowing for more biologically-based risk estimates for specific nickel compounds.

The current epidemiological data are not sufficient to fully address whether the nickel ion theory or the nickel ion bioavailability model is more appropriate for predicting lung cancer risks. In most cases, workers were exposed to several forms of nickel, making it impossible to tease out which particular nickel form was associated with risk.

In the few cohorts in which exposures were to one form of nickel alone, these exposures were generally small, leading to studies that were statistically underpowered for the assessment of risk. It does not appear that these limitations can be overcome in future studies. Thus, it is not likely that epidemiological data will ever be sufficient to fully address which model is the most appropriate for determining the carcinogenicity of nickel-containing substances.

11. Conclusions

The nickel ion theory purports that exposure to any nickel-containing substance leads to an increased cancer risk that is proportional to that substance's water solubility. Although the epidemiological evidence is not robust regarding the carcinogenicity of various forms of nickel, the available animal and mechanistic data do not support the nickel ion theory; rather, they are supportive of the nickel ion bioavailability model. The nickel ion bioavailability model is a refinement of the nickel ion theory and holds that a nickel-containing substance must release nickel ions that then become bioavailable at the nucleus of epithelial respiratory cells for the substance to be a complete carcinogen, and that the carcinogenic potency of the substance is proportional to the degree to which the nickel ions are bioavailable. The bioavailability depends on the respiratory toxicity, clearance, intracellular uptake, and both the extracellular and intracellular dissolution of a particular nickel-containing substance. These factors lead to the different carcinogenic potencies of various nickel-containing substances in animal and perhaps epidemiological studies.

The nickel ion bioavailability model best describes the mechanistic data for nickel-containing substances. These data indicate that sulfidic nickel exposures lead to higher nuclear bioavailability of nickel ions, whereas exposures to copper-free nickel oxides, water-soluble nickel compounds, and metallic nickel lead to lower nuclear bioavailability of nickel ions. This is consistent with the results of animal studies in which nickel subsulfide caused the highest tumor induction in rats (NTP, 1996b), copper-free nickel oxide induced tumors in rats only at much higher exposure levels, under conditions of impaired particle clearance (NTP, 1996c), and both water-soluble nickel and nickel metal did not induce tumors at the MTD (NTP, 1996a; Oller et al., 2008). This is also consistent with epidemiology studies showing clear associations with lung cancers for sulfidic nickel and nickel-copper oxides and no consistent associations for nickel oxides that do not contain copper or for water-soluble nickel compounds.

Some regulatory groups are working to incorporate more mechanistic data into hazard and risk assessment. The International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO) has published a framework for evaluating the mode of action for chemical carcinogenesis in experimental animals (Sonich-Mullin et al., 2001) and has recently updated this framework to address the relevance of a cancer mode of action in animal studies to humans (Boobis et al., 2006). The use of mode-of-action information in the assessment of potential carcinogens is also a main focus of US EPA's current *Guidelines for Carcinogen Risk Assessment* (US EPA, 2005). The Scientific Committee on Occupational Exposure Limits (SCOEL) in the European Union (EU) considers mode-of-action data in risk assessments that inform the setting of occupational exposure limits for carcinogens (Bolt and Huici-Montagud, 2008). Information on animal toxicity is being collected for many substances under the new REACH chemical policy in the EU, and guidance documents for REACH discuss the importance of addressing the underlying mechanism of action and considering information such as the physicochemical properties of a substance when choosing the appropriate route of exposure in hazard and safety assessments (ECHA, 2008). The use of the nickel ion bioavailability model could improve the hazard identification process for both tested and untested nickel-containing substances if knowledge of respiratory toxicity, clearance, intracellular uptake, and the extracellular and intracellular dissolution are known. Information on bioavailability is necessary for the assessment of the carcinogenic risks of nickel-containing substances, and should be incorporated into future risk assessments.

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